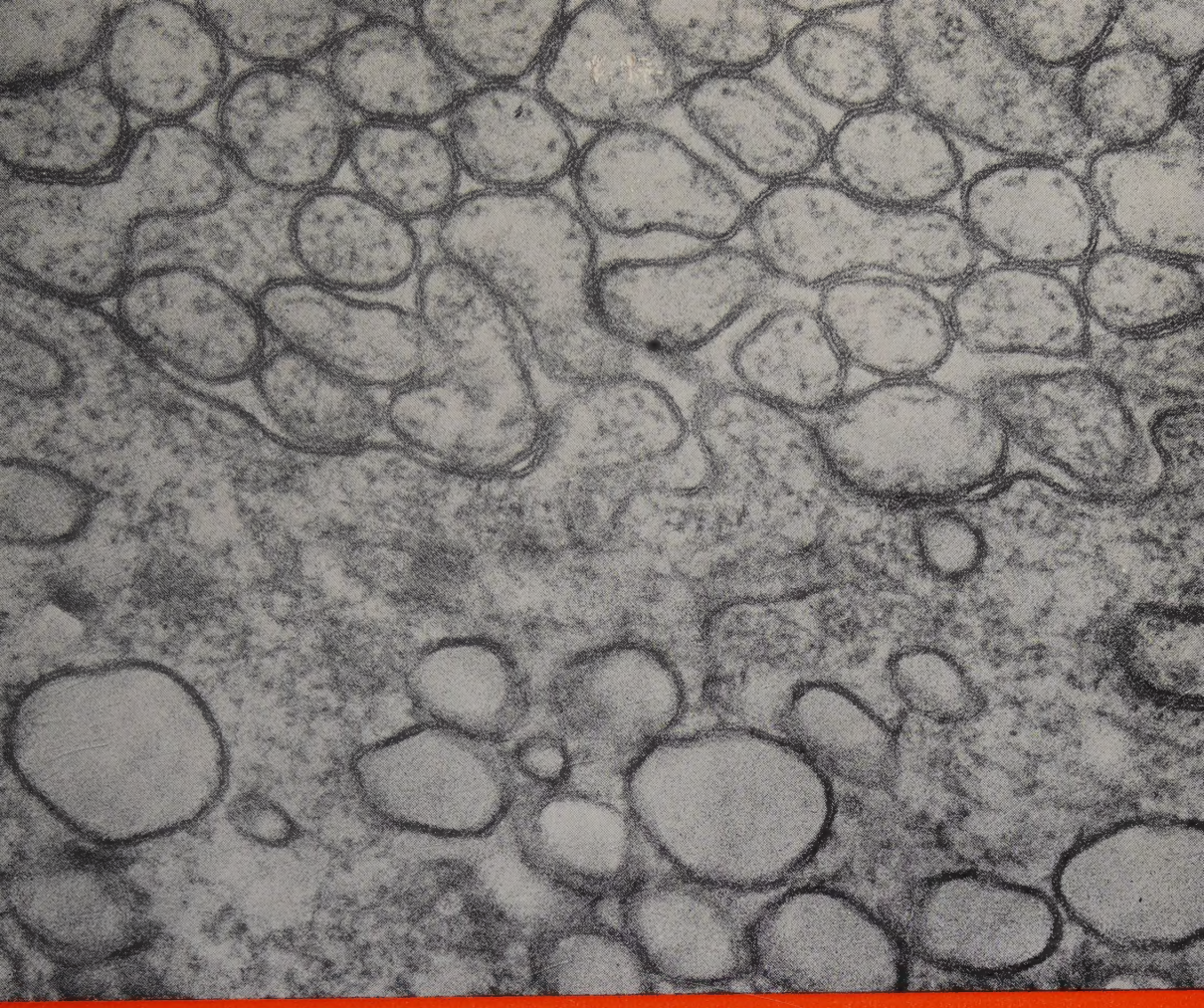

# Ultrastructure of Fundus Glands of the Mouse Gastric Mucosa

## An Electron Microscopical Study in Fasted and Refed Animals with Observations on Ultrastructure after Different Fixation and Embedding Procedures

HERBERT F. HELANDER

Journal of **Ultrastructure Research**

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BY

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UPPSALA 1962

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# GENERAL INTRODUCTION

Ever since the discovery, at the beginning of the nineteenth century, that gastric juice contains hydrochloric acid, the various cells of the gastric mucosa have been studied in a large number of investigations. At the end of that century, when these cells had been identified with the aid of the light microscope, attention was transferred to those changes which take place in the light microscopic appearance of the gastric mucosa when its activity is stimulated. The basic physiological experiences of the mode of reaction of the gastric mucosa—with which the name of Pavlov is associated—date from the same period. In recent decades the gastric mucosa has also been studied from a biochemical point of view, especially with respect to the energy metabolism and the contents of enzymes in the various epithelial cells.

Notwithstanding these intense researches, a number of important problems remain unsolved as regards the function and the reactions of the gastric mucosa. For example, although a lively debate has been going on for many years concerning the mechanism underlying the production of hydrochloric acid by the gastric mucosa, it still has not been definitely established in which type of cell the hydrochloric acid is produced. In addition, a number of highly topical clinical problems urgently need to be solved. What causes the high incidence of cancer in the stomach? Which mechanism is responsible for the onset of peptic ulcer? How is the gastric mucosa involved in the production of substances capable of preventing pernicious anemia? A better knowledge of the ultrastructure of the gastric mucosa may serve to bring one or more of the biological problems of this tissue nearer solution.

The aim of the present investigation was to study under different functional conditions the ultrastructure of the fundus glands of the gastric mucosa, and to correlate the observations made with previously established morphological, physiological, and biochemical data. In view of the rapid post-mortal decomposition of the gastric mucosa—known also in light microscopy—and the consequent difficulty of preserving this tissue in a manner adequate for electron microscopic studies, trials have also been made with different methods of fixation and embedding.

# PART I. FASTED MICE

## CHAPTER 1

## Survey of the Literature

### ABSTRACT OF LIGHT MICROSCOPIC OBSERVATIONS

A good many authors have described at length the histology of the mammalian gastric mucous membrane. The following is a summary of the most characteristic light microscopic features of the various epithelial cells of the fundus glands as they appear in the majority of mammals. This summary is based on monographs (*3*, *9*, *165*), modern textbooks (*5*, *125*), articles (*27*, *43*, *44*, *200*), and personal observations.

The corpus region of the gastric mucosa, in the mouse ranging in thickness from 0.3 to 0.5 mm, lodges tightly packed glands, the so-called fundus glands or, as they are also known, chief glands. A fundus gland may be divided into three portions which, reckoned from the deepest layer of the gastric mucous membrane, are: the base (the body), the neck, and the isthmus. The glands open in the pits (the crypts) on the surface of the gastric mucosa.

The basal part of the fundus glands is dominated by *zymogen cells*. They are characterized by their content of large granules, so-called zymogen granules, and by the basophilic staining reaction of their basal portions. The zymogen cells are thought to elaborate the pepsin of the gastric juice.

In the neck and in the base, as well as in the isthmus, one encounters *parietal cells*. They are often larger than the other epithelial cells of the fundus gland and frequently situated peripherally to these cells relative to the glandular lumen. Their outstanding characteristics are the intracellular canaliculi which open at the apical surface of the cells. Though conclusive proof is lacking, the parietal cells are held responsible for the hydrochloric acid production of the fundus glands.

All the cells at the surface of the gastric mucosa and in the pits, as well as some of those in the isthmi, are *surface epithelial cells*. They are cuboidal or cylindrical

mucus-producing cells characterized by an accumulation of PAS-positive granules in the apical portion of the cytoplasm. Whereas those surface epithelial cells which are situated in the lower portion of the isthmus contain comparatively few granules, those in the upper portion of the isthmus and in the pit contain a markedly larger number, whilst the proportion of these granules once again is smaller at the surface of the gastric mucosa.

The necks are the site of the so-called *mucous neck cells*, most of which are cuboidal in shape. PAS staining imparts a foamy, pink appearance to the entire cytoplasm of these cells. Gradual changes between mucous neck cells and surface epithelial cells are said to occur. Most probably the mucous neck cells also elaborate mucus.

*Argyrophil cells*, lastly, are encountered only rarely in the fundus glands of the mouse. The majority are found in the basal part of the glands. These cells are distinguished by containing an abundance of granules stainable with certain silver compounds and, moreover, by their position out of contact with the glandular lumen. The argyrophil cells are presumed to have an endocrine function.

*A note on the nomenclature*

Among the many appellations given to the cells of the fundus glands, the ones used here were selected because they express something about the properties of the respective cells. However, in order to prevent misconceptions, it seems appropriate to enumerate some commonly encountered synonyms for the various cells.

The *zymogen cells* have also been called chief cells (*Hauptzellen*, *Grundhauptzellen*, *cellules principales*), adelomorphous cells, central cells, chief cells of the body, pepsinogen cells, peptic cells, and zymogenic cells.

The *parietal cells* (*Belegzellen*, *cellules bordantes*), have been designated acid cells, delomorphous cells, and oxyntic cells.

The *surface epithelial cells* (columnar cells, *Oberzellen*) and the *mucous neck cells* (neck chief cells, *Halshauptzellen*, *Nebenzellen*, *Zwischenzellen*, *cellules principales muqueuses*) have certain staining reactions in common and are therefore often discussed under the common heading of *mucoid cells* (*180*).

Considering that the nature has not been established of the relations between the argentaffin and argyrophil cells (cf. *27*, *89*), the more inclusive term *argyrophil cells* will be adopted here. Cells which probably were identical with the argyrophil cells have also been designated basal clear cells, chromaffin cells, enterochromaffin cells, yellow cells (*basal-gekörnte Zellen*, *helle Zellen*, *cellules argento-réductrices*); in addition they have received a number of eponyms including Heidenhain, Nussbaum, Nicholas, and Kultschitzky cells.

## ELECTRON MICROSCOPIC LITERATURE

The fundus glands of the mammalian gastric mucosa have also been subjected to electron microscopical studies. In so doing most workers have confined themselves to the ultrastructure of the parietal cells. Only a few papers on the electron microscopical appearance of other epithelial cells in these glands have been published. The results obtained will be summarized in the following.

With the aid of the electron microscope, a variety of workers have examined fundus-gland cells in mouse (*24*, *34*, *35*, *37*, *74–78*, *84–87*, *98*, *99*), rat (*98*, *99*, *109*, *115*, *182–184*, *191*, *204*, *206*), guinea pig (*128*), bat (*99*, *100*), rabbit (*202*), hamster and beaver (*98*, *99*), cat (*98*, *99*, *183*, *184*, *204*), kitten (*99*), dog (*98*, *99*, *148*, *183–187*, *202*, *204*), and man (*23*, *73*, *99*, *120*, *121*, *128*, *203*).

No systematic investigation has been published on differences in the ultrastructure of the fundus-gland cells between different mammalian species. However, judging from the information given in the literature no major differences seem to exist. Similarly Vial *et al.* (*204*) noted only minor dissimilarities in the ultrastructure when comparing parietal cells of the dog, the cat and the rat. Consequently, the cells will here be treated without regard to their origin.

### *Zymogen cells*

The apical *plasma membrane* of the zymogen cells bounds a number of microvilli (*34*, *35*, *37*, *84*, *86*, *87*, *109*, *128*, *191*). The plasma membranes on the lateral surfaces are on the whole straight, apart from occasional interdigitations with adjacent epithelial cells (*109*, *128*, *148*). A *terminal bar* is found near the transition to the apical surface (*109*). The course of the basal plasma membrane is essentially straight (*86*, *109*).

A prominent feature of the zymogen cell is the large number of *zymogen granules* encountered mainly in the apical region of the cytoplasm (*84*, *109*, *128*, *191*). These round or oval formations have a homogeneous (*86*), dense (*86*, *87*) content and are demarcated by a distinct membrane (*86*). However, the content of the zymogen granules is often less dense (*86*, *87*, *108*, *109*, *128*, *148*, *191*) and has a finely granular appearance (*34*, *86*, *109*). Such zymogen granules often have an incomplete surface membrane (*86*, *109*, *191*).

Moreover, the zymogen cells contain a small number of oval or elongated *mitochondria* (*84*, *86*, *87*, *109*, *128*), often situated close to the lateral cell surfaces (*109*, *191*). They have a number of transversely disposed inner membranes (*86*, *109*).

One further observes in the zymogen cell cytoplasm an extensive *Golgi apparatus* (*86*, *87*, *109*, *191*) composed of membranes, vacuoles, and vesicles (*86*, *109*, *191*). A few zymogen granules are present in the neighborhood of the Golgi apparatus (*86*, *87*, *109*, *191*).

*α-Cytomembranes* are encountered mainly in the basal half of the cell (*34*, *84*, *86*, *108*, *109*, *128*, *191*) where they form long parallel aggregations (*84*, *86*, *108*, *109*, *191*). Apically there occur shorter, often circularly disposed α-cytomembranes, limiting cisternae of varying size (*109*).

*Vacuole-containing bodies* (*78*, *84*) and *dense microbodies* (*84*, *115*) are other, more rarely occurring cytoplasmic components.

The *nucleus*, encountered in the basal portion of the zymogen cell, is generally round or oval (*109*). It is equipped with a three-layered surface membrane (*86*, *109*, *148*), on the external surface of which small, dense particles are visible (*109*). Discontinuities have been seen in the surface membrane of the nucleus (*206*). Occasionally the outer layer of the surface membrane is observed to be continuous with an α-cytomembrane (*109*).

*Parietal cells*

The parietal cells are often located peripherally to the other epithelial cells in relation to the glandular lumen, with which they then communicate by way of an intercellular canal (*74*, *84*, *109*, *204*). On the apical cell surface, which is provided with numerous *microvilli* (*34*, *35*, *37*, *73*, *84*, *86*, *87*, *99*, *109*, *115*, *128*, *204*) emerge the characteristic *intracellular canaliculi* (*37*, *73*, *74*, *78*, *86*, *87*, *99*, *100*, *108*, *109*, *115*, *128*, *182*, *186*, *187*, *203*, *204*), which penetrate deeply into the cytoplasm. Microvilli also abound along these canaliculi (*24*, *34*, *35*, *37*, *73*, *74*, *78*, *84*, *86*, *87*, *99*, *100*, *109*, *115*, *120*, *128*, *148*, *182*, *183*, *185–187*, *202–204*). Occasionally branched (*99*), the microvilli are bounded by a three-layered *plasma membrane* (*74*, *84*, *86*, *115*, *204*) and contain a finely granular dense substance (*73*, *84*, *115*, *186*, *187*), also described as an additional membrane underlying the plasma membrane (*74*, *78*).

On the apical cell surface several workers have also observed major cytoplasmic processes containing a few vesicles and a finely granular substance of low density (*99*, *108*, *109*, *115*, *191*). However, cells with such formations display signs of poor preservation (*115*).

The lateral plasma membranes are, apart from minor interdigitations with adjacent epithelial cells (*73*, *109*, *115*, *182*, *203*), more or less straight. However, microvilli are said to project from the lateral cell surfaces into intercellular canaliculi which have been found to separate some of the parietal cells from their neighbors (*115*). Near the transition to the apical cell surface one regularly encounters a *terminal bar* (*109*, *115*). The basal cell surface exhibits a number of small infoldings (*73*, *74*, *86*, *99*, *109*, *115*, *182*, *203*) bridged by the basement membrane (*73*, *74*, *99*, *115*).

In the cytoplasm there are a number of round or oval *vacuoles*, an observation reported in all papers published after 1954. They are particularly prevalent near the intracellular canaliculi (*73*, *74*, *78*, *108*, *109*, *115*, *203*) and in the apical portions of the

cell (*204*). They are bounded by a smooth-surfaced membrane (*73*, *74*, *78*, *108*, *109*, *115*, *120*, *148*, *183–187*). Moreover, some workers have reported findings suggesting that vacuoles communicate with the glandular or canalicular lumen (*78*, *109*).

In addition to these vacuoles, tubules have also been found (*98*, *99*, *115*, *186*, *187*), the diameter of which is considerably less than that of the vacuoles (*115*). Some of these tubules are said to be intimately associated with mitochondria (*115*) or to merge with the apical cell surface (*98*), and occasionally vacuoles and tubules appear to be interconnected (*115*, *186*, *187*). Parietal cells containing tubules rather than vacuoles have been observed too (*98*, *99*).

A large number of round or oval or even branched (*73*, *108*, *109*) *mitochondria* have been noticed by all workers who have studied the ultrastructure of parietal cells. Mitochondria are particularly numerous in a perinuclear zone (*74*, *115*), in the neighborhood of the intracellular canaliculi (*73*), and in a marginal zone bordering on the basal (*115*, *204*) and lateral (*115*) cell surfaces. They contain a large number of closely spaced inner membranes (*24*, *73*, *74*, *78*, *84*, *86*, *99*, *108*, *109*, *115*, *128*, *148*, *182*, *183*, *185–187*, *191*, *203*, *204*), which sometimes curve, branch or anastomose (*115*), and a dense mitochondrial matrix (*73*, *74*, *78*, *84*, *86*, *99*, *109*, *115*, *186*, *187*, *203*, *204*) together with a few small, dense particles (*24*, *73*, *99*, *115*, *187*). Some authors have claimed that attachments exist between some mitochondria and the lateral plasma membrane (*109*), mutually between mitochondria, and between mitochondria and vacuoles, α-cytomembranes or the nuclear membrane (*73*), but the resolution of the published electron micrographs is so low that they cannot be accepted as proof of such a statement.

Only some of the investigators have mentioned the existence of a *Golgi apparatus* in the parietal cells (*24*, *73*, *99*, *100*, *115*, *182*, *187*, *191*, *203*). Poorly developed, it consists of membranes, vacuoles, and a few vesicles (*73*, *187*, *191*). It has been proposed that the numerous cytoplasmic vacuoles are components of the Golgi apparatus (*76*), a hypothesis previously rejected by others (*109*).

The parietal cell cytoplasm is also the site of quite few, small, dense *particles* which tend to be more common in the basal part of the cell (*115*, *203*) and near the nucleus (*109*, *115*, *203*). Sometimes they are observed near short membranes (*73*, *74*, *86*, *99*, *100*, *109*, *115*, *120*, *182*, *183*, *187*, *203*) thus composing α-cytomembranes, but often they are free, collected into small groups in the cytoplasm (*73*, *74*, *84*, *86*, *87*, *99*, *186*, *187*).

A section from the central parts of a parietal cell will usually include one or more so-called *vacuole-containing bodies* (*73–76*, *78*, *84*, *87*, *115*, *186*, *187*, *191*, *204*) which appear as round formations demarcated by a smooth membrane and containing a number of small vacuoles (*75*, *78*, *115*, *204*).

*Dense microbodies* are encountered about as often as vacuole-containing bodies.

The dense microbodies are round or oval formations, usually smaller in size than the mitochondria (*115*), and consist of irregularly grouped, dense and less dense areas (*84*, *115*).

*Lipid droplets* (*73*, *76*), *lamellar or granular cytosomes* (*73*), *granules* about 400 Å in diameter (*186*, *187*), and *tonofibrils* (*73*, *115*) are other rarely occurring cellular organelles.

The *nucleus* of the parietal cell is round (*73*, *86*, *109*, *115*) or oval (*109*) and lies centrally (*74*, *100*, *115*) or basally (*73*) in the cell. It has a three-layered surface membrane (*73*, *86*, *109*, *115*, *182*) in which pores are observed (*182*, *206*). The outermost layer of the nuclear membrane is studded with a few small, dense particles (*73*, *109*, *115*) and is reported occasionally to be continuous with an α-cytomembrane (*74*). Internally the nucleus is occupied by a finely granular substance (*73*) which, near the nuclear margin and in occasional central regions (*73*, *86*, *109*), is packed more closely than elsewhere in the karyoplasm.

*Surface epithelial cells*

The apical *plasma membrane* of the surface epithelial cell demarcates a small number of irregularly shaped cytoplasmic processes of varying size (*34*, *37*, *84*, *86*, *100*, *109*, *128*, *148*). The lateral plasma membrane is occasionally highly convoluted (*84*, *86*, *108*, *109*, *115*, *128*, *148*). Sometimes it forms microvilli projecting into dilatations of the intercellular space (*191*). Basally the plasma membrane is undulated (*109*).

In the apical cytoplasm one finds a large number of closely packed, dense *secretory granules* of varying shape (*84*, *86*, *100*, *108*, *109*, *128*, *191*). Many secretory granules are reported to have a denser core (*191*).

The *mitochondria* are round or rod-shaped (*84*, *86*, *109*) and contain only a few inner membranes (*86*). Almost the entire mitochondrion is often occupied by a round, dense granule (*84*, *86*).

Of the various epithelial cells of the fundus gland, the surface epithelial cell is said to possess the most extensive *Golgi apparatus* (*109*). Very often it is encountered in the supranuclear portion of the cytoplasm (*84*, *109*) or in the paranuclear zone (*109*). It is composed of essentially straight-running membrane pairs in the neighborhood of which numerous small granules and vesicles are present. These granules vary greatly in size (*109*).

*α-Cytomembranes* occur sparsely (*84*, *86*, *108*, *109*, *148*); a large number of small, dense *particles* are scattered throughout the cytoplasm (*84*, *86*, *108*), perhaps more abundantly in the basal part of the cell (*109*); *dense microbodies* may also be encountered in these cells (*115*).

The *nucleus* exhibits a round (*86*) or oval (*109*, *128*) shape with an irregular contour (*108*, *109*, *191*) and is surrounded by a three-layered surface membrane (*86*, *109*).

*Mucous neck cells*

Little has been published on the ultrastructure of the mucous neck cell. Judging by published electron micrographs and reported results, Kurosumi's (*108*), Kurosumi's *et al.* (*109*), and Shibasaki's (*191*) descriptions of the ultrastructure of the mucous neck cell would seem to be more applicable to less well preserved zymogen cells.

Apparently surface epithelial cells and mucous neck cells exhibit great ultrastructural similarities (*84*, *100*). Attempts have been made to distinguish mucous neck cells from surface epithelial cells on the basis of differences in the amount and distribution of secretory granules, in the appearance of the lateral cell surfaces, and in the incidence of large, dense, intramitochondrial granules (*84*).

The *plasma membrane* on the apical surface of the mucous neck cell bounds a small number of short microvilli (*34*, *84*, *148*). The other cell surfaces are smooth (*84*), although the lateral surfaces reportedly have interdigitations with adjacent epithelial cells (*148*).

In the supranuclear portion of the cytoplasm of the mucous neck cell *secretory granules* and *Golgi structures* have been observed (*84*, *148*). Further organelles encountered in these cells include *mitochondria*, short bundles of *α-cytomembranes* (*84*, *148*), numerous dense *particles*, *vacuoles*, *vacuole-containing bodies* (*84*), and occasional *dense microbodies* (*115*).

*Argyrophil cells*

The surface of the argyrophil cells nowhere abuts on the glandular lumen (*85*, *109*, *191*). The *plasma membrane* of this cell has a fairly straight course (*85*, *109*).

A particularly characteristic feature of the argyrophil cells is the presence of a large number of *granules* dispersed throughout the cytoplasm (*85*, *100*, *109*, *191*). A granule consists of a dense core separated from the surface membrane by a less dense zone (*85*, *148*).

The *mitochondria* are round or elongated (*85*, *108*, *109*) and have a small number of inner membranes (*85*).

In the argyrophil cell one sometimes further encounters a small number of irregularly shaped, *opaque areas*, measuring 1 to 2 $\mu$ across and bounded by a varying number of concentric membrane pairs (*85*).

The presence has also been reported in the cytoplasm of a *Golgi apparatus*, numerous *vacuoles*, a number of small, dense *particles* (*85*, *108*, *109*), *filaments* (*109*, *148*), and *lipid droplets* (*109*).

The *nucleus*, which lacks distinctive features, is bounded by a three-layered surface membrane (*85*).

*Other, less well-defined cells*

Little information is available on other cell types in the fundus glands. A special *neck parietal cell* found in the neck of the fundus gland and believed to be an immature prestage of the parietal cells has been described (*115*).

In the neck region another type of cell has been described which is devoid of secretory granules but contains a large number of dense particles. This type of cell has been interpreted as a young, proliferating cell which will replace other epithelial cells of the gland (*191*).

## CHAPTER 2

# Materials and Methods

### EXPERIMENTAL ANIMALS

Female albino mice aged from 3 to 5 months and weighing between 18 and 35 g at the time of death were used as experimental animals. Because an epizootic occurred during the experimental period two different strains of mice had to be used. The animals lived under normal laboratory conditions in glass jars in a room with a temperature around +25°C. They had free access to water and food,[1] except during a 36 hour period of strict starvation immediately before sacrifice, when they received nothing but water. To preclude coprophagia during the starvation period, the mice were put into individual Perspex tubes measuring 10 cm by 3 cm and equipped with transverse rods in either end to prevent escape (Text-fig. 1).

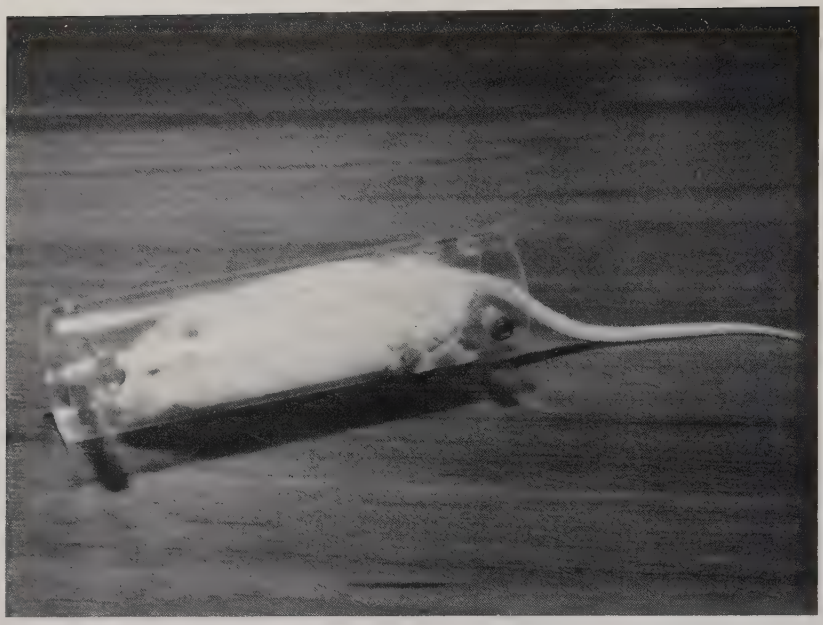
### TISSUE PREPARATION

At the end of the starvation period the mice were killed. In the hope of avoiding the effect of any diurnal variations all the mice were sacrificed between 9 a.m. and noon. The animals were killed by decapitation. A portion of the corpus region of the stomach along the greater curvature was excised with a pair of scissors. The excised tissue was immersed in ice-cold fixation fluid and then subdivided with scissors into pieces of approximately 1 $mm^3$. The delay between decapitation and such immersion never exceeded 2 minutes. The whole procedure was carried out at a room temperature of about +1°C.

The problem of achieving satisfactory preservation of the tissue was attacked in several ways. In the first place trials were made to facilitate the *penetration of fixation fluid* into the tissue. This was attempted by perfusion of the animal with the fixation fluid immediately after decapitation (cf. *211*), via the aorta, the coeliac trunk or the portal vein, after which fixation of the tissue was continued as described above. Alternatively a small amount of fixation fluid was injected between the muscularis

[1] Composition of the Swedish Cooperative Society's Mouse Bread, parts by weight:

| | | | |
|---|---|---|---|
| Lean dehydrated milk | 3.00 | Lucerne flour | 10.00 |
| Bread crumbs | 47.71 | Beef meal | 2.50 |
| Oatmeal fodder | 21.00 | Fish liver oil | 0.04 |
| Wheat germs | 15.75 | Total | 100.00 |

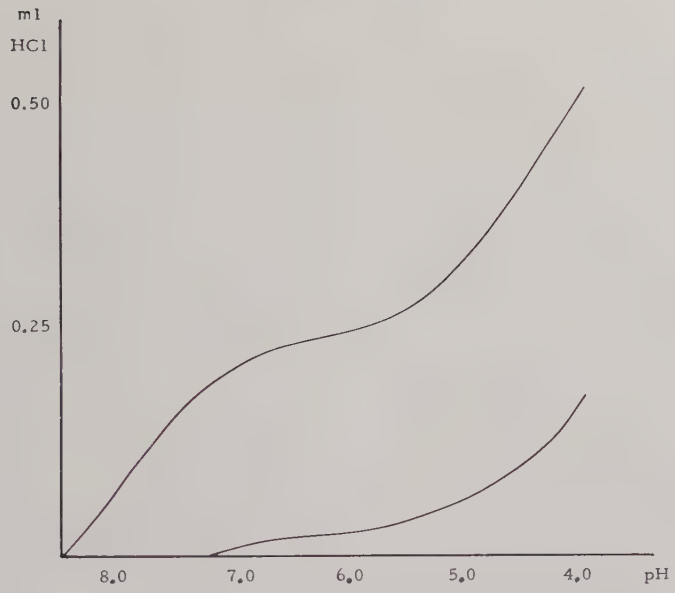


TEXT-FIG. 1. Design of cages in which the fasting mice were kept to prevent coprophagia.

and the mucosa of the gastric wall (in accordance with Vial *et al.* (*208*)). Since these procedures apparently did not improve the quality of the preservation, they were later abandoned.

In the second place the *pH and buffering capacity of the fixation fluid* were altered. Alternatively to the commonly used pH of 7.2, the pH of the fixation fluid was raised to 8.5 and at the same time the buffering capacity was increased (Text-fig. 2). The composition of the two veronal acetate buffered (*132*) fixation fluids, the first of which was prepared according to earlier recommendations (*150*, *192*, *211*), was as follows:

| pH 7.2 | | pH 8.5 | |
|---|---|---|---|
| Veronal sodium | 0.613 g | Veronal sodium | 1.349 g |
| Sodium acetate | 0.405 g | Sodium acetate | 0.890 g |
| Sodium chloride | 0.431 g | Sodium chloride | 0.009 g |
| 0.10 *N* hydrochloride acid | 24.0 ml | Calcium chloride | 0.111 g |
| Redistilled water to make | 100.0 ml | 0.10 *N* hydrochloride acid | 11.5 ml |
| wherein was dissolved | | Redistilled water to make | 100.0 ml |
| Osmium tetroxide | 1.0 g | wherein was dissolved | |
| | | Osmium tetroxide | 1.0 g |




TEXT-FIG. 2. Titration curves for the two fixation fluids (osmium tetroxide omitted) used. In both cases, 1.0 *N* HCl was added to a 10.0 ml sample consisting of 5.0 ml fixation fluid to which had been added an equal volume of redistilled water, the top curve representing fixation fluid buffered to pH 8.5 and the lower one that buffered to pH 7.2. The fixation fluid of pH 8.5 evidently has an appreciably higher buffering capacity towards acidity. (The curves were recorded on a Radiometer "Titrator", Type TTT 1b, equipped with a "Titrigraph", Type SBR 2a.)

Both these fixation fluids showed a freezing point depression of 0.55°C (determined mainly according to the Beckmann method (cf. *198*)) and were consequently isotonic to blood (*119*).

In the third place different *embedding media* were employed. Thus either a methacrylate mixture (*13*, *140*) or a polyester, Vestopal W[1] (*179*), was used.

The tissue specimens were fixed, dehydrated, infiltrated, and embedded according to the following scheme:

| Methacrylate | Vestopal |
|---|---|
| *Fixation*: 1½ hours in ice-cold fixation fluid. | *Fixation*: 1½ hours in ice-cold fixation fluid. |
| *Rinsing*: 1 hour in 0.94% sodium chloride solution. | *Rinsing*: 1 hour in 0.94% sodium chloride solution. |

[1] Supplied by M. Jaeger, Vésenaz, Geneva, Switzerland.

| Methacrylate | Vestopal |
|---|---|
| *Dehydration*: 1 hour in each of 70, 85, 96, and 100% alcohol. | *Dehydration*: 20 minutes in each of 30, 50, 75, and 90% acetone. Dehydration: 60 minutes in two changes of 100% acetone. |
| *Infiltration*: 12 hours at +4°C in a monomeric methacrylate mixture containing 15% methyl methacrylate and 85% butyl methacrylate to which had been added 0.5% benzoyl peroxide. | *Infiltration*: 2 hours at +20°C in 1 part Vestopal and 2 parts acetone, 2 hours in 2 parts Vestopal and 1 part acetone, and 12 hours in Vestopal alone. |
| *Embedding* in gelatin capsules containing a prepolymerized methacrylate mixture of the same composition as above. | *Embedding* in gelatin capsules containing Vestopal to which had been added 1% activator,[1] 1% initiator,[1] and, as plasticizer, 3% dibutylphtalate. |
| *Polymerization*: 24 hours at +60°C. | *Polymerization*: 24 hours at +60°C. |

A total of 60 mice were used in Part One of this investigation. Pieces of gastric mucosa from the same animals were often preserved in different ways. Thus pieces of gastric mucosa from 53 of these animals were fixed at pH 7.2 and from 15 at pH 8.5, and subsequently embedded in methacrylate. Tissue specimens from 16 of the mice were fixed at pH 8.5 and then embedded in Vestopal. For comparative purposes fundus glands fixed at pH 7.2 and embedded in Vestopal from two of the animals were also examined.

The morphological observations were made on this material after exclusion of poorly preserved specimens (cf. p. 74). Thus after fixing at pH 7.2 and embedding in methacrylate only seven of the 53 animals could be the basis of the morphological observations presented in Chapter 3, the remaining 46 being discarded because of poor preservation. In the other groups of differently fixed and/or embedded specimens no animal was excluded. In total the morphological observations were based on 25 mice.

## SECTIONING

When the embedded specimen was being trimmed under the dissection microscope, the level at which sectioning of the fundus glands appropriately should commence could, not seldom, be roughly estimated because of the fact that the gastric wall of the mouse is clearly divided into a muscular portion and a three times thicker mucosal portion. As a rule the sections were cut parallel to the mucosal surface.

[1] Supplied by M. Jaeger, Vésenaz, Geneva, Switzerland.

After trimming, the specimens were sectioned, either on a microtome designed by Ekholm and Zelander (*54*) or on an Ultrotome (*88*) by LKB-Produkter, Stockholm, Sweden. At first stainless steel knives (*52*) and later glass knives (*114*) were used.

The sections were floated and allowed to stretch for 20 minutes on a 20% dioxane solution and were then picked up on a copper grid provided with a Formvar film.

To intensify the contrast of the structures in methacrylate embedded sections, some of these were immersed for 1 hour in a saturated aqueous solution of uranyl acetate at +20°C. Excess uranyl acetate was removed from the grids by a brief rinse in 0.1 *N* acetic acid solution and then in redistilled water. All Vestopal sections were similarly stained for 1½ hours and rinsed.

## ELECTRON MICROSCOPY

Three electron microscopes—an EMU Model 3b by RCA, a Tronscope Model TRS50 by Akashi and an Elmiskop I by Siemens—were employed in the present investigation. The RCA instrument was equipped with a 250 $\mu$ condensor aperture and the Akashi microscope with a 50 $\mu$ anode aperture. Most of the time the Siemens microscope was used with both condensor lenses, the aperture of the second condensor lens having a diameter of 100 $\mu$. All the microscopes had objective pole pieces well compensated for astigmatism and objective apertures 50 $\mu$ in diameter. The accelerating voltage was 50 kV in the RCA and Akashi microscopes and 60 kV in the Siemens instrument. The electron optical magnification ranged from 1000 to 45,000 times and was calibrated against a shadow-cast carbon replica of a diffraction grating having 28,800 lines per inch (E. F. Fulham Inc., Schenectady, N.Y., U.S.A.). Electron micrographs were recorded on Ilford N50 plates and developed in Ilford's Caustic Hydroquinone Developer.

## MEASUREMENTS AND STATISTICS

The dimensions of structures were determined by means of a magnifier, having a scale with 0.1 mm divisions, on 3–5 times enlarged paper prints of well-focused negatives. No distances on the paper prints shorter than 0.25 mm were measured in order to keep within reasonable limits the percentage error in reading the scale (cf. *211*).

Only structures possessing a distinct outline were measured. Consequently the diameter of zymogen granules with a discontinuous surface membrane was measured between clearly defined points on portions of unbroken surface membrane at the

ends of a diameter. The data from these measurements are compiled in Table I (pp. 114–116).

Certain frequently occurring structures were selected for statistical analysis of the variation of their dimensions after different preservation methods. When testing whether the dimensions of a formation differed significantly in these cases, standard statistical procedures were employed (cf. *33*). The level of significance was set at 5 %. The means and standard deviations are presented in Table II (p. 117).

## LIGHT MICROSCOPICAL CONTROL

In order to preclude the possibility that the stomachs which were studied electron microscopically were affected with pathological changes, 10 $\mu$ sections from the remainder of the gastric mucosa from the corpus region of each mouse were examined under the light microscope after fixation in 5% neutralized formalin solution and staining with hematoxylin eosin, mucicarmine, or with the periodic acid-Schiff procedure of McManus.

## CHAPTER 3

# Results

*A note on the nomenclature*

Among the various surfaces of the epithelial cells of the fundus gland, that bordering against the glandular lumen will here be called *apical*, those facing adjacent epithelial cells *lateral*, and that adjoining the basement membrane *basal*. The term paranuclear is used to designate the cytoplasm occupying the zone between the supranuclear and infranuclear cell portions.

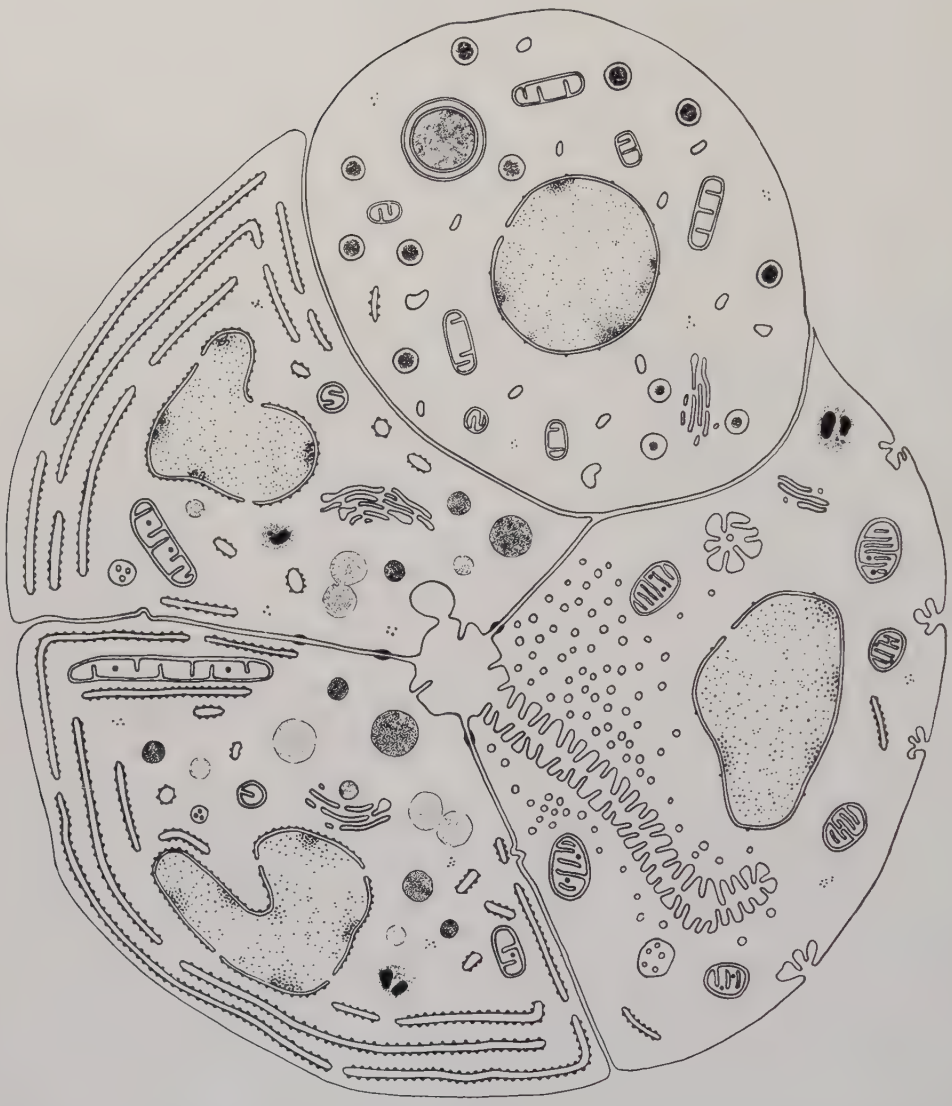
The electron optical density varies considerably between different cellular structures. This may be due to differences in the composition of the tissue itself, or in the osmium content (cf. 7). No attempts were made to measure the density differences. However, the author has tried to differ between the densities observed by using the following terms: "maximally dense", "of high density", "dense" (synonym "opaque"), "moderately dense", "of low density", and "of the same density as the embedding medium". It must be emphasized that this subdivision of the different densities observed on the electron micrographs should not be strained, since estimation of degrees of density are subjective.

Since the electron micrographs in the present study did not reveal any peculiarities capable of serving as criteria for distinguishing the surface epithelial cells from the mucous neck cells, these two cells will be described and discussed under the common heading of *mucoid cells*. Consequently, in the present study it has not been feasible sharply to demarcate the surface epithelium of the gastric mucosa from that of the fundus glands.

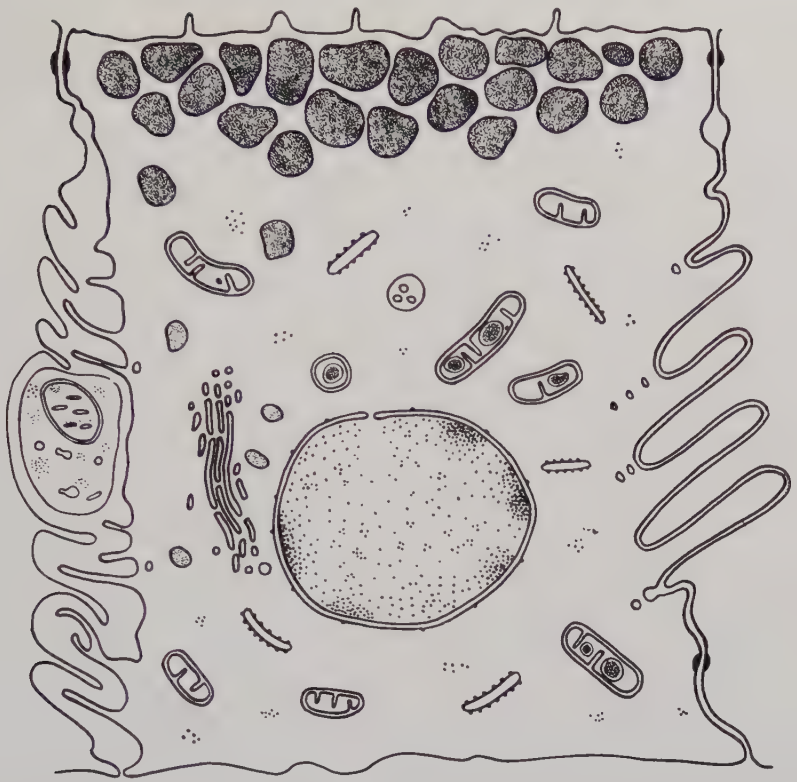
## ULTRASTRUCTURAL ORGANIZATION OF THE FUNDUS GLANDS

Features which are well known from light microscopy were on the whole borne out by the present investigation. The fundus glands are tubular with the long axis roughly perpendicular to the mucosal surface. The smallest tubular diameter is of the order of 25 $\mu$.

The *walls* of a glandular tubule are composed of the secretory cells, in cross-sections usually numbering from 3 to 10. Most of these cells are shaped like a sector of a cylinder with the tip truncated to make room for the glandular lumen. However,



TEXT-FIG. 3. Schematic representation of a fundus gland in cross-section, as usually manifested in fasted mice. The glandular lumen is in the centre. To the left there are two zymogen cells containing, among other things, nuclei, long $\alpha$-cytomembrane strands, mitochondria, Golgi apparatus, and zymogen granules of varying density. To the right is a parietal cell on whose apical surface opens an intracellular canaliculus lined by numerous microvilli. In the cytoplasm of the parietal cell there are large mitochondria with many inner membranes, a great number of vacuoles, a vacuole-containing body, a dense microbody, and a comparatively small Golgi apparatus. The argyrophil cell near the top of the diagram does not abut on the glandular lumen. Its cytoplasm contains a nucleus, a pigment body, secretory granules, vacuoles, mitochondria and a Golgi apparatus.



TEXT-FIG. 4. Schematic representation of a mucoid cell. Immediately beneath the apical cell surface there are a large number of closely packed secretory granules. In addition, the cytoplasm is the site of mitochondria with intramitochondrial granules, a Golgi apparatus, and numerous small particles. The intercellular space to the left is widened and contains disintegrating organelles. The plasma membrane to the right is markedly undulated, forming interdigitations with the adjacent cell. Rows of vacuoles are shown which seem to have been pinched off from invaginations of the lateral cell surface.

other shapes are not seldom encountered, especially among those peripheral parietal cells which lie displaced from the lumen by the other epithelial cells of the gland. Owing to their small apical surface, some of these parietal cells seem to lack contact with the glandular lumen in sections cut in unfavorable directions. In cross-sections of the fundus glands such parietal cells are shaped more or less like a circle segment. The argyrophil cells are round or oval and never border on the lumen.

The *lumen* runs approximately down the midline of the fundus gland (Fig. 1). In sections deemed roughly perpendicular to the long axis of the gland, the lumen mostly has a round or oval general shape and a minimum diameter of about 1 $\mu$. Occasional slot-shaped lumina are also encountered.

Generally the lumen contains a substance of low density. In various places, however, one encounters small, indistinctly bounded patches of an unstructured or finely granular, dense substance. These tend to be particularly numerous in the vicinity of the apical surfaces of the zymogen and mucoid cells (Figs. 3, 6, 7, 15, 25). More extensive dense regions are only seldom encountered within the lumen of the fundus glands (Fig. 25). Occasionally the lumen in methacrylate embedded specimens is wholly or partly filled by cytoplasmic blebs bulging from the apical surfaces of the parietal cells (Fig. 13). However, such appearances were invariably accompanied by signs of poor preservation and have never been observed in tissue fixed at pH 8.5.

Basally the glandular cells border against the *basement membrane*, the continuous investment with which each separate gland is provided. The basement membrane is an indistinctly defined, homogeneous, moderately dense structure whose thickness varies between approximately 150 and 400 Å (Figs. 4, 13, 15, 17, 18, 23, 26, 27, 35, 36, 42).

## ULTRASTRUCTURE OF THE SECRETORY CELLS OF THE FUNDUS GLANDS

In the following will be described the ultrastructure of the epithelial cells of the fundus glands, such as it appears in sections from well-preserved tissue specimens fixed at pH 7.2 and embedded in methacrylate. The observations will then be compared with corresponding findings derived from methacrylate embedded specimens fixed at pH 8.5. Lastly an account will be given of the electron microscopical appearance of the fundus glands fixed at pH 8.5 and embedded in Vestopal.

### Methacrylate Embedded Tissue Fixed at pH 7.2

*Zymogen cells*

Zymogen cells are encountered chiefly in the basal part of the fundus glands where they constitute the main component of the glandular walls. The zymogen cells border against the lumen apically, against adjacent epithelial cells laterally, and against the basement membrane basally.

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Fig. 1. Cross section through the upper part of a fundus gland, where it consists of mucoid cells exclusively. The slot-like glandular lumen (*L*) is situated in the centre of the gland. In the cytoplasm near the lumen there are a number of moderately dense secretory granules (*G*). The maximally dense granules are intramitochondrial. Several cell nuclei (*N*) and Golgi apparatus (*GA*) are seen. The presence of small, cytoplasmic particles in large numbers imparts a fairly high overall density to the cells. Methacrylate embedded section fixed at pH 7.2. × 6500.

N
GA
N
G
L
N

Projecting from the apical surface of the zymogen cell and bounded by its *plasma membrane*, there are a small number of finger-like cytoplasmic processes which henceforth will be referred to as *microvilli* (Figs. 2, 6, 7). They have an average diameter of some 780 Å and maximal length of about 0.2 $\mu$. Next to the transitions towards the lateral cell surfaces, the apical cell surface is often provided with cytoplasmic projections which are shorter and broader than the microvilli (Fig. 2). Otherwise the apical plasma membrane is fairly straight. It is distinctly three-layered with a dense layer on either side of a less dense layer, each layer being about 25 Å thick (Figs. 6, 7).

Along the lateral cell surfaces the plasma membrane takes a fairly straight course (Figs. 2, 3, 11, 39), but in various places it outlines short cytoplasmic processes, some of which are intertwined with corresponding processes on adjacent cells (Figs. 2, 39). In the following these formations will be referred to as *interdigitations*. The plasma membrane on the lateral cell surfaces has a thickness of about 50 Å.

The *intercellular space* separating a zymogen cell from an adjacent epithelial cell has a fairly constant width of about 80 Å, except in the neighborhood of interdigitations where it tends to widen. Where the apical and lateral cell surfaces meet, the middle of the intercellular space is occupied by a dense *stratum* measuring some 20 Å in thickness and usually exhibiting a length of about 0.2 $\mu$. Occasionally it can be seen how this structure apparently is formed through fusion of the outer layers of the apical plasma membranes of two adjacent zymogen cells (Fig. 7).

Close to their junction with the apical cell surface, the lateral cell surfaces regularly have a *terminal bar* (Figs. 2, 7). In the sections this feature appears as a thickening about 0.2 $\mu$ long of two facing plasma membranes and a concomitantly increased density of the neighboring cytoplasm. The plasma membranes are often seen to have a three-layered structure in these regions too.

Formations resembling terminal bars are not seldom observed elsewhere along the lateral plasma membranes of adjacent zymogen cells, although in the sections they seem to have only about one-third of the apico-basal extent of the terminal bars (Figs. 2, 7). They will be called *desmosomes* in the following.

The plasma membrane on the basal cell surface is on the whole straight (Figs. 4, 5).

The large granules found in the supranuclear and paranuclear cytoplasm—here-

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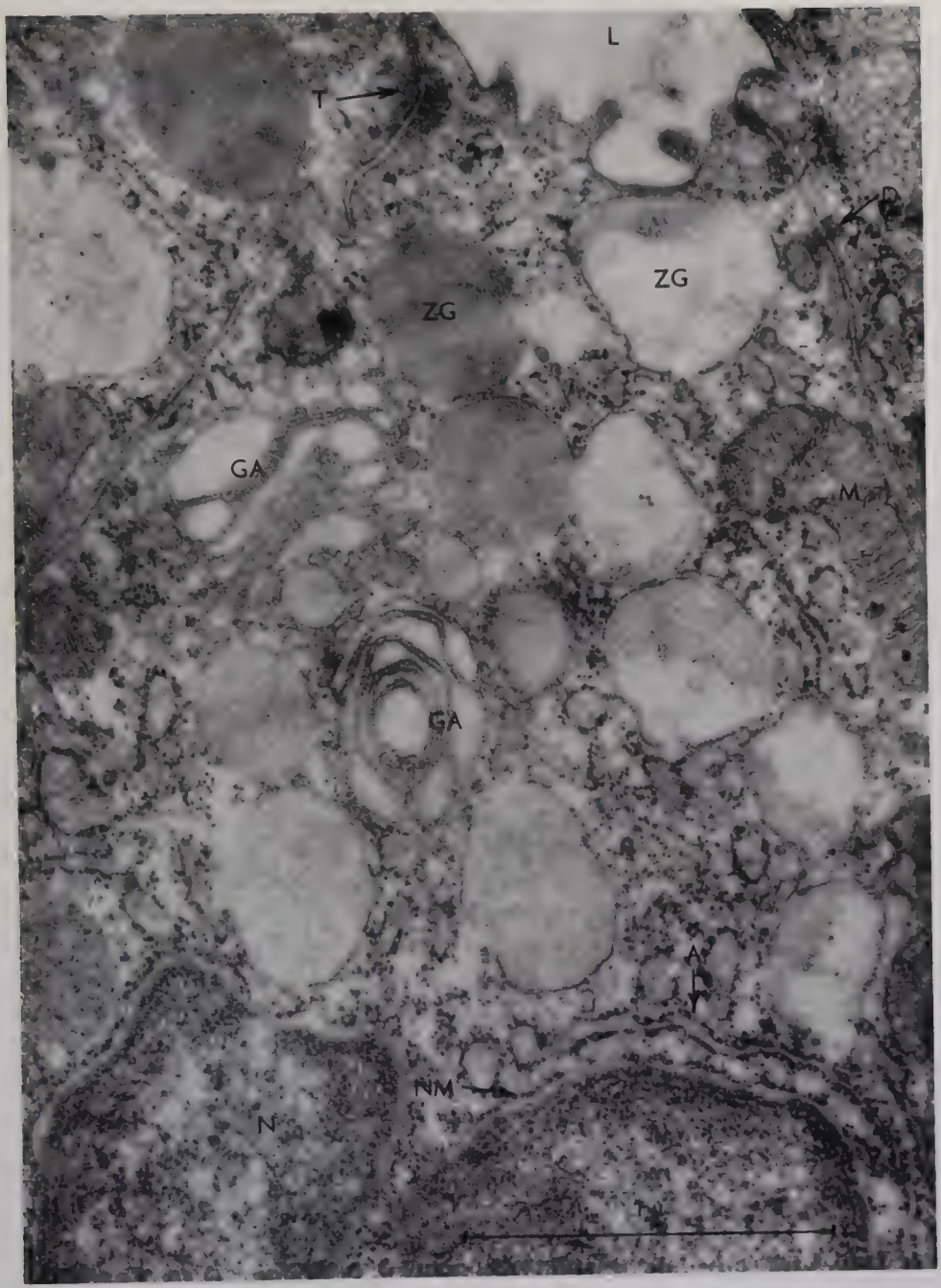
FIG. 2. Supranuclear part of a zymogen cell. From its apical surface short microvilli project into the glandular lumen (*L*). Both terminal bars (*T*) and desmosomes (*D*) are present along the lateral surfaces. Zymogen granules (*ZG*) of varying density are seen, those in the neighborhood of the Golgi apparatus (*GA*) being smaller than those elsewhere. A few irregularly disposed strands of α-cytomembranes (*A*) are visible. The nucleus (*N*) has a three-layered surface membrane (*NM*). Mitochondria (*M*) occur sparsely. Methacrylate embedded section fixed at pH 7.2. × 50,000.


L
T
ZG
ZG
GA
M
GA
A
NM
N

after termed *zymogen granules*—are typical of the zymogen cell (Figs. 2–5, 7, 10, 11). With a round or oval shape and a mean diameter of 0.70 $\mu$, the zymogen granules have either a homogeneous and dense or a somewhat floccular and moderately dense content. In the latter case their content sometimes is unevenly distributed, and their surface membrane—the thickness of which is of the order of 60 Å—is incomplete (Fig. 2). As a rule both types of zymogen granule are present in the same cell. Cells containing solely the moderately dense type of zymogen granules almost always display signs of poor preservation. Zymogen granules are not seldom situated so close together that their surface membranes seem to touch (Figs. 2, 3, 40). Contiguous zymogen granules with incomplete surface membrane frequently give the impression of being confluent (Figs. 2, 3, 7). The apical portion of the cytoplasm sometimes exhibits formations resembling the moderately dense type of zymogen granule. These formations are demarcated from the surrounding cytoplasm by a distinct membrane which, however, merges imperceptibly with the apical plasma membrane. Hence the contents of such regions are continuous with the contents of the glandular lumen (Figs. 3, 7).

*Mitochondria* are less abundant in zymogen cells than in other fundus-gland cells (Figs. 2, 3, 5, 7, 40). Apparently they are not confined to any particular cytoplasmic region. On electron micrographs they are round, oval or elongated and have transversely disposed inner membranes (Figs. 2, 3, 11, 12). The minor mitochondrial axis averages 0.29 $\mu$, and the measured maximum length is about 4 $\mu$. Along the greater part of the mitochondrial outline the finely undulating outer membrane is composed of three layers, a less dense layer about 50 Å thick being interposed between two dense layers about 30 Å thick. The mitochondrial inner membranes, of a similar three-layered appearance, are sometimes continuous with the mitochondrial outer membrane, the less dense layer of the latter then merging with the less dense layer of the former. Whereas the dense layers of the inner membranes are about 30 Å thick, their less dense intermediate layer is about 70 Å thick. The distance between the mitochondrial inner membranes ranges from approximately 150 to 1000 Å. The mitochondrial matrix is moderately dense and generally contains a few round, homogeneous, opaque particles measuring some 290 Å across. These particles exhibit no topographical relationship to the inner membranes (Fig. 11).

A *Golgi apparatus* is often present in one or more supranuclear or paranuclear

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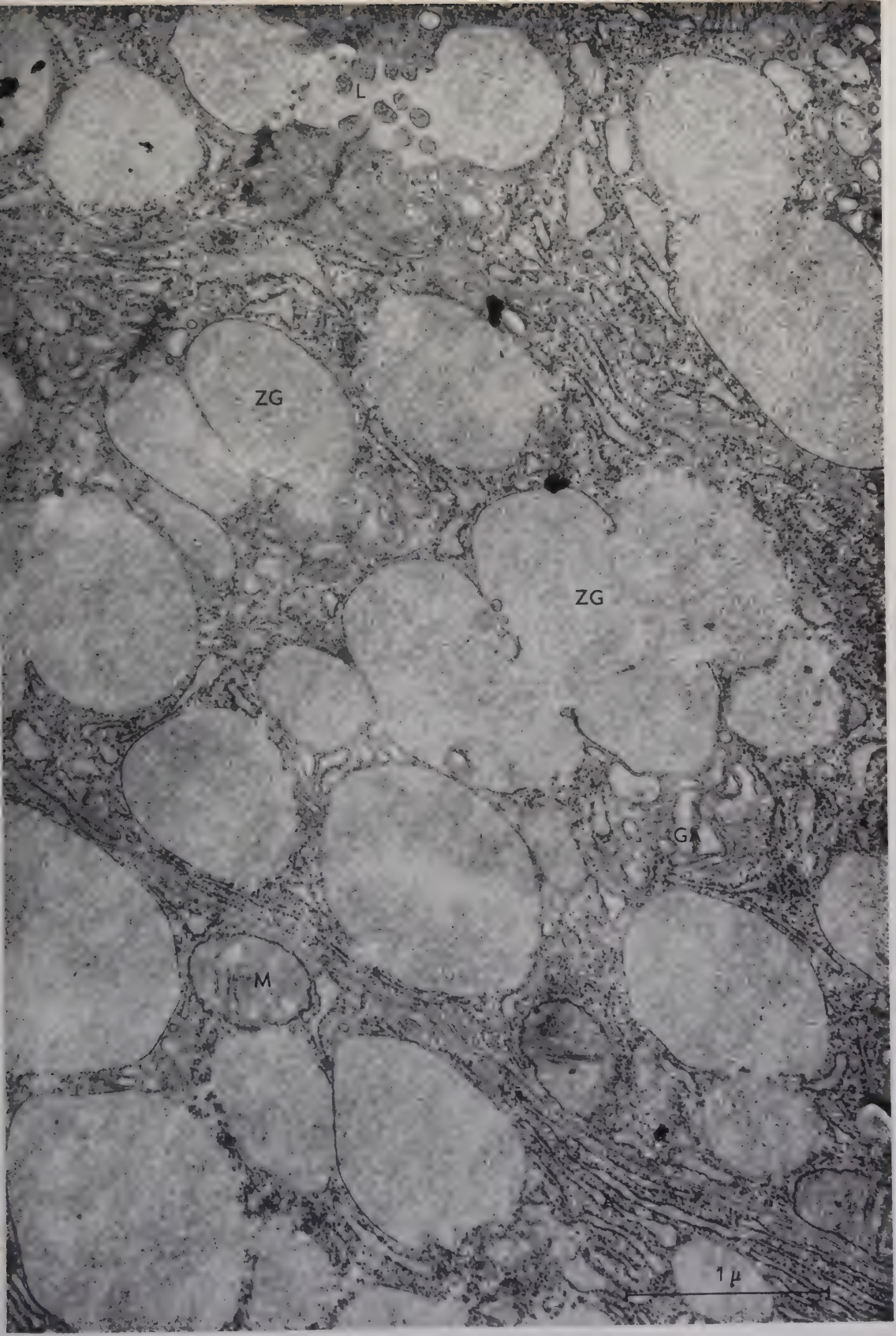
FIG. 3. Supranuclear part of a zymogen cell. The zymogen granules (*ZG*) contain a finely granular, moderately dense substance and many have an incomplete surface membrane. Not seldom contiguous zymogen granules seem to coalesce. It appears that two zymogen granules are discharging their contents into the glandular lumen (*L*). *A*, α-cytomembrane; *GA*, Golgi apparatus; *M*, mitochondrion. Vestopal embedded section fixed at pH 8.5. ×34,000.


L
ZG
ZG
M
1 μ

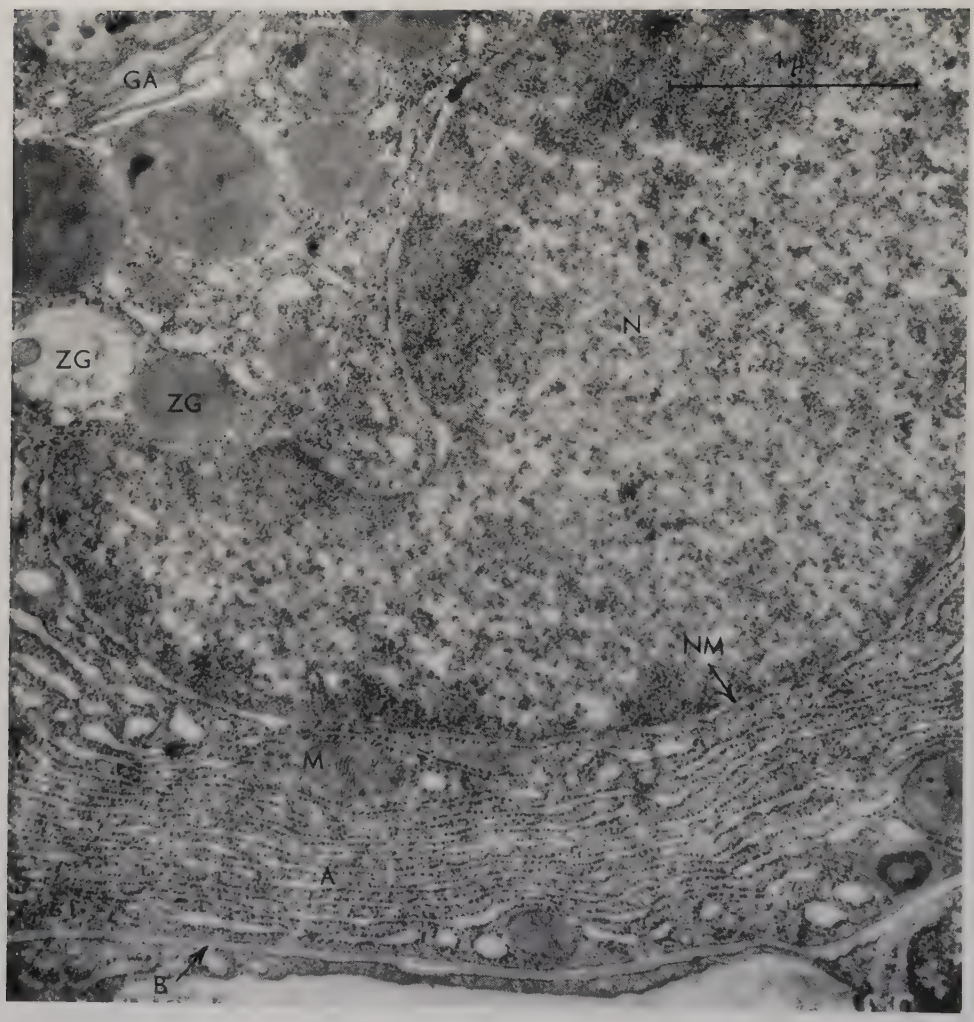
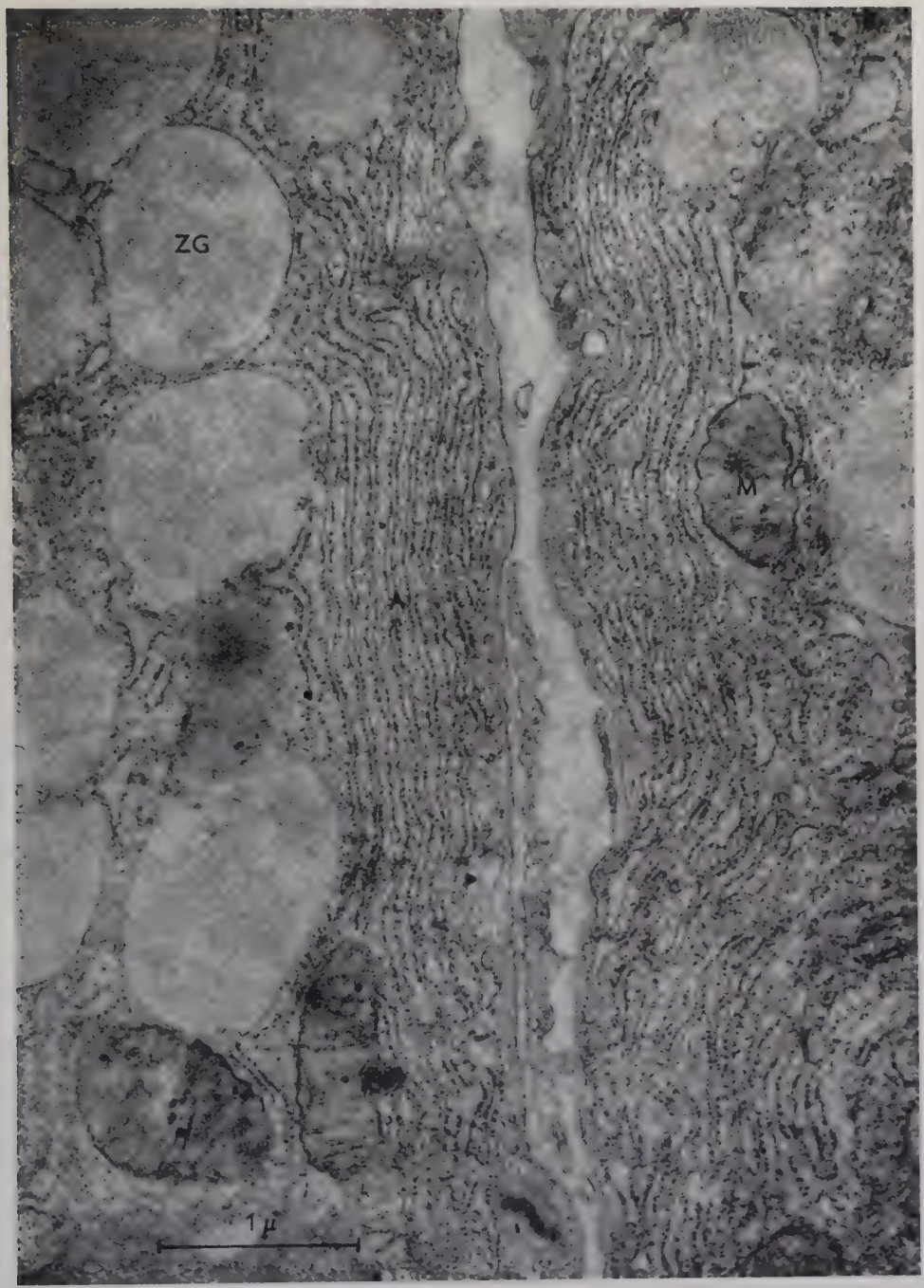



FIG 4. Basal part of a zymogen cell. Here the α-cytomembranes (*A*) are very regularly disposed, roughly parallel to the basal cell surface. Between their particle-bearing surfaces there is a large number of free, maximally dense particles, occasional zymogen granules (*ZG*) and mitochondria (*M*). The nucleus (*N*) has a three-layered surface membrane (*NM*). The basement membrane (*B*) is visible beneath the basal plasma membrane. *GA*, Golgi apparatus. Methacrylate embedded section fixed at pH 7.2. ×32,000.

FIG. 5. Basal portions of two zymogen cells, with several α-cytomembrane pairs (*A*), zymogen granules (*ZG*) and mitochondria (*M*). Vestopal embedded section fixed at pH 8.5. × 28,000.


ZG
M
1 μ

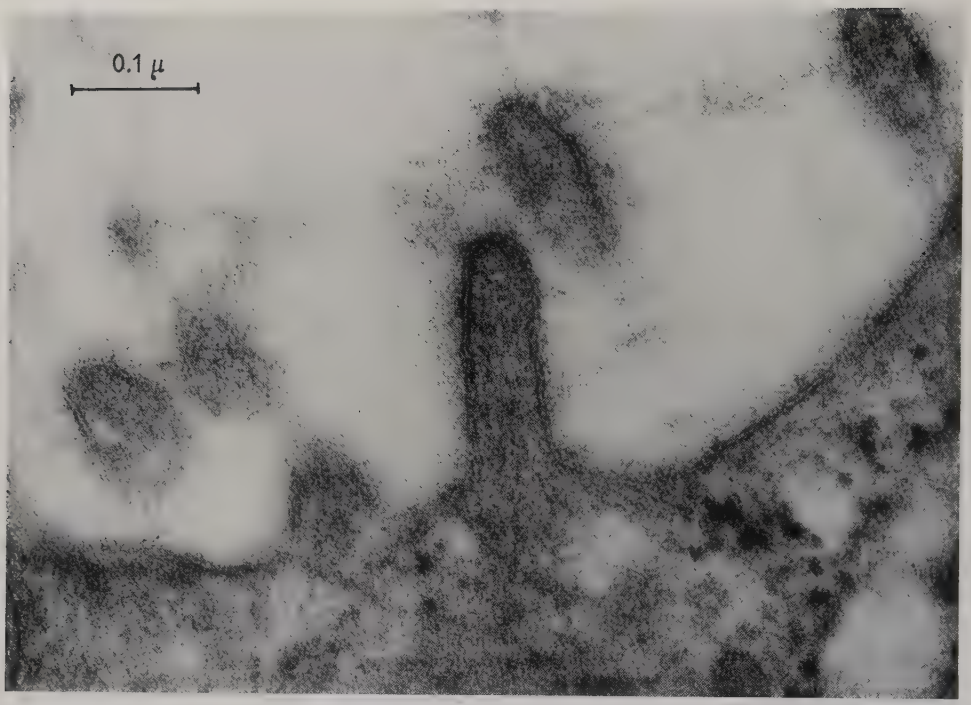
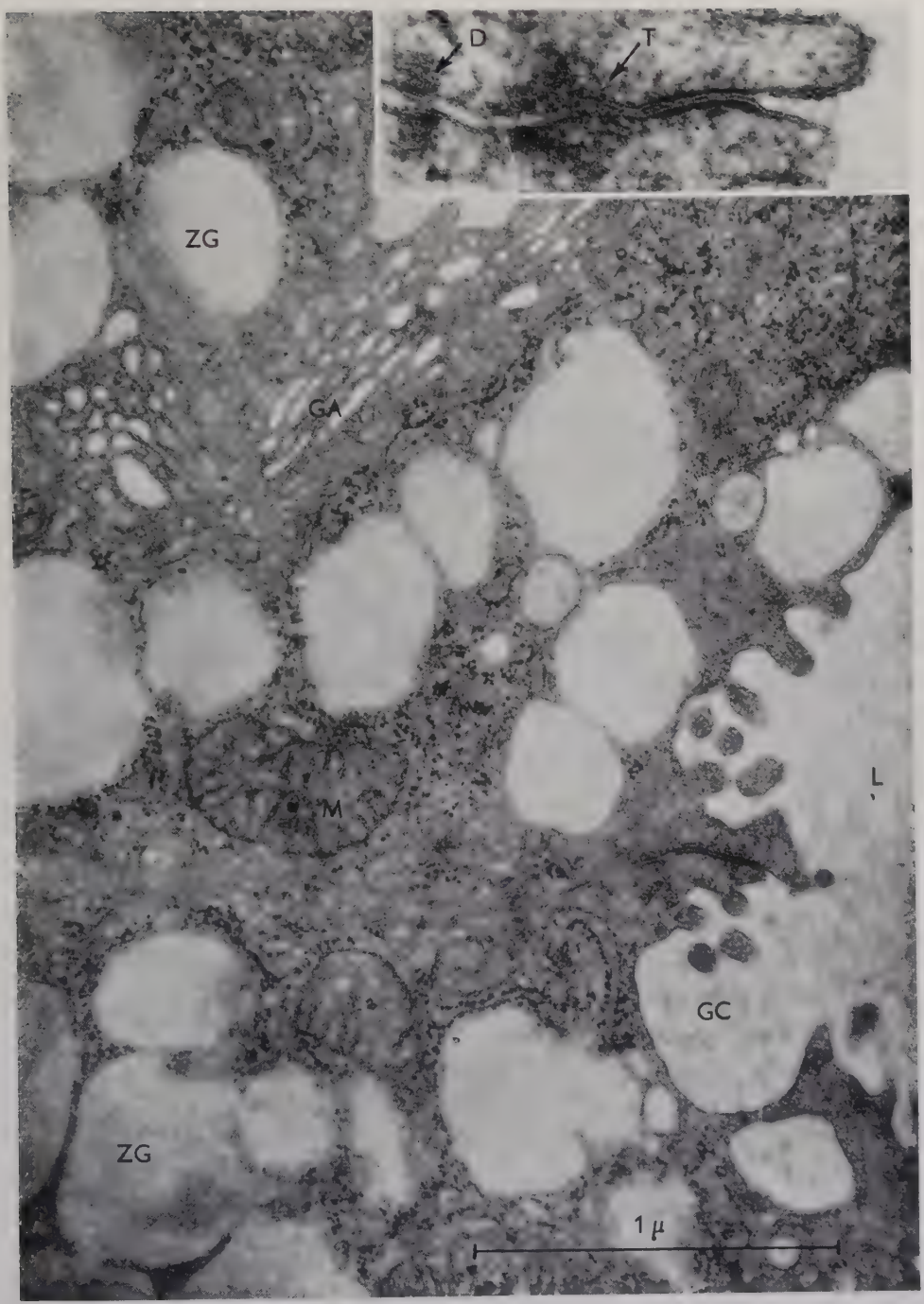



FIG. 6. Detail of the most apical portion of a zymogen cell and adjacent portions of the glandular lumen. One longitudinally and several obliquely sectioned microvilli are seen. The plasma membrane is distinctly three-layered. Small patches of dense material are present in the glandular lumen, especially in the immediate vicinity of the cell surface. Methacrylate embedded section fixed at pH 7.2. × 170,000

regions of the cytoplasm. As a rule they comprise 6 to 12 membranes, a few vacuoles and a comparatively small number of vesicles (Figs. 2, 3, 7, 10). Averaging 50 Å in thickness, the membranes are disposed in pairs which for varying portions of their length are parallel and some 70 Å apart. At both ends of such a parallel portion the two membranes of a pair diverge and become continuous with the adjacent membranes of the next membrane pairs. The region thus enclosed by the membranes from two adjacent pairs constitutes a so-called Golgi vacuole. Such vacuoles are of highly

FIG. 7. Apical part of two zymogen cells. The zymogen granules (*ZG*) are as a rule of uniformly low density, although some exhibit areas of higher density. To the right it appears that the contents of a zymogen granule (*GC*) is continuous with the glandular lumen (*L*). The dense cytoplasm is the site of mitochondria (M), a Golgi apparatus (*GA*), and numerous small particles. Methacrylate embedded section fixed at pH 8.5; magnification: × 50,000. Inset shows the terminal bar region (*T*) of two neighboring zymogen cells; a desmosome (*D*) is also visible. The intercellular space contains a thin, dense stratum, apparently formed through fusion of the two outer layers of the three-layered plasma membranes. Methacrylate embedded section fixed at pH 7.2, × 120,000.


D
T
ZG
GA
M
L
GC
ZG
1 μ

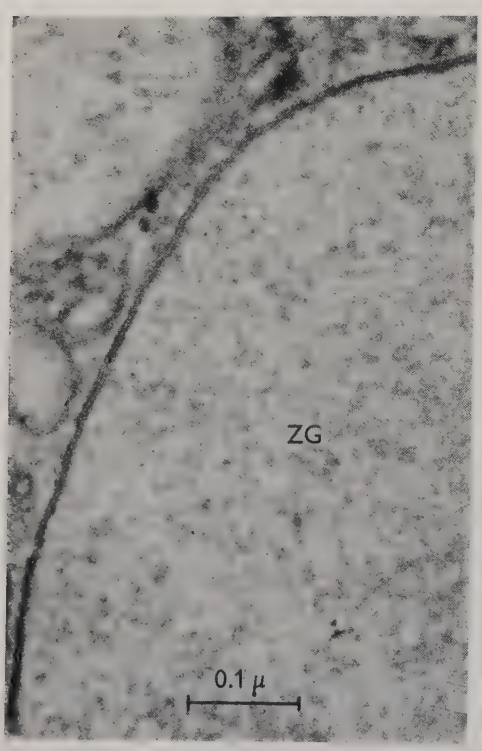



FIG. 8.

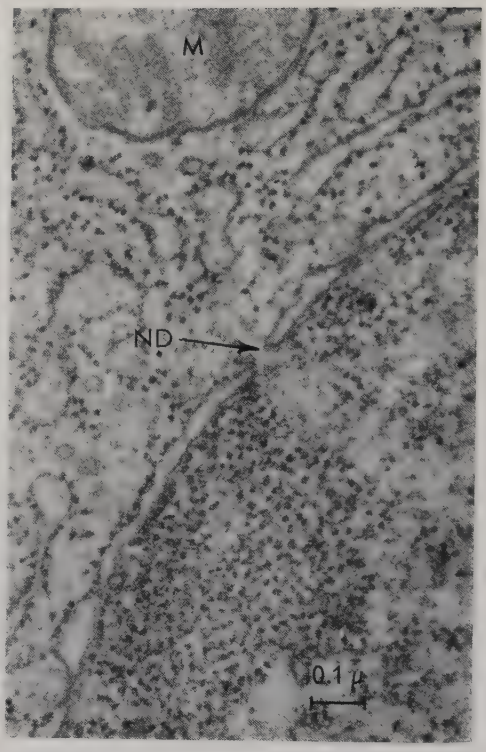



FIG. 9.

FIG. 8. Detail of a zymogen granule (*ZG*). The three-layered appearance of its surface membrane is clearly visualized. Vestopal embedded section fixed at pH 8.5. ×150,000.

FIG. 9. Part of the nucleus of a zymogen cell, the adjacent cytoplasm containing α-cytomembranes and a mitochondrion (*M*). The nuclear membrane exhibits a discontinuity (*ND*) beneath which the karyoplasm contains a reduced number of maximally dense particles. Vestopal embedded section fixed at pH 8.5. ×68,000.

varying shape and size, their largest dimension seldom exceeding 1 μ. They contain a substance which usually is homogeneous and of the same density as the embedding medium but may be rather floccular and of higher density. The round or oval Golgi vesicles are situated peripherally to this system of membranes and vacuoles. Their longest diameter seldom exceeds 0.1 μ. They contain a homogeneous, moderately dense substance. There are also in the neighborhood of the Golgi apparatus a large number of zymogen granules which are more polymorphic than those elsewhere in the cell (Figs. 2, 10). Round or oval, they vary greatly in size but are usually smaller than other zymogen granules. Zymogen granules in the vicinity of the Golgi apparatus almost always have a complete surface membrane. The inner substance is finely

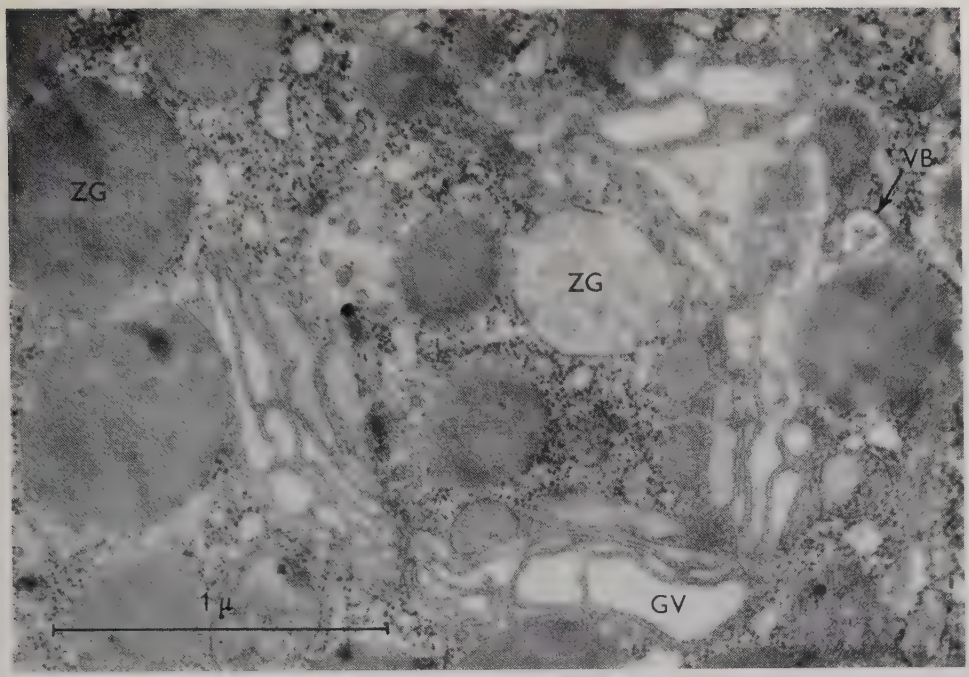



FIG. 10. Golgi apparatus in a zymogen cell. It is composed of membranes bounding Golgi vacuoles (*GV*). No Golgi vesicles are present. Some zymogen granules (*ZG*) of varying density are seen close to the Golgi apparatus. *VB*, vacuole-containing body. Methacrylate embedded section fixed at pH 7.2. ×43,000.

floccular and of low density in some and homogeneous and much more dense in others; although all stages intermediate between these are seen.

Particle-bearing membranes—*α-cytomembranes* according to Sjöstrand (*193*)—dominate in the infranuclear portion of the cytoplasm (Figs. 4, 5). They take the form of paired membranes, approximately 60 Å thick and of varying length, disposed roughly parallel to the cell surface. Those surfaces of a membrane pair which face away from one another are studded with a great number of round or polygonal, maximally dense *particles* whose diameter averages about 130 Å. The membranes of a pair are fused at the ends and hence enclose elongated sacks or cisternae about 200 Å wide. Although it may vary within wide limits, the distance between the particle-bearing surfaces of regularly disposed α-cytomembranes usually attains some 400 Å. α-Cytomembranes which are found outside the infranuclear regions of the cytoplasm are less regularly organized, and the cisternae—here appreciably smaller—most often have a round or oval configuration (Fig. 2). The cisternae of the zymogen cells

contain a homogeneous substance of low density, where neither zymogen granules nor other organelles are encountered. Between the particle-bearing surfaces of the α-cytomembranes a large number of free particles are found which have the same appearance and dimensions as those attached to the membranes. In these regions of the cytoplasm other organelles are also found such as mitochondria and zymogen granules.

A few *vacuole-containing bodies* are present in the supranuclear cytoplasm of the zymogen cells (Figs. 10, 11). Round or oval with a diameter of about 0.24 μ, these formations are bounded by a distinct surface membrane and generally contain from 3 to 8 vacuoles scattered in a homogeneous ground substance of the same density as the embedding medium. The mean vacuolar diameter is about 380 Å.

Occasionally this cytoplasmic region also contains *dense microbodies* of varying size. These round or oval formations have a diameter seldom exceeding 0.5 μ and an inner structure which varies greatly in appearance. Generally, however, they exhibit a few rounded, maximally dense patches separated by a less dense substance.

The dense microbodies are readily distinguishable from the generally homogeneous formations of high density occasionally seen in the basal portion of the zymogen cells. These formations, henceforth called *lipid droplets*, usually have an irregularly indented outline, and their diameter may exceed one μ. As a rule both dense microbodies and lipid droplets lack distinct surface membranes.

The *ground cytoplasm*, apparently without structure, is moderately dense, except in a narrow marginal zone near the apical plasma membrane where the density is appreciably higher than elsewhere in the cell (Figs. 2, 6).

As a rule situated in the basal portion of the cell, the *nucleus* generally is of round or oval shape with a smooth margin, although nuclei with deep indentations also were seen (Figs. 2, 4). The nucleus is bounded by a three-layered surface membrane consisting of a dense layer (each 60 Å) on either side of a less dense (300 Å) layer. The cytoplasmic surface of the external layer is studded with small, maximally dense particles. At intervals the nuclear membrane exhibits discontinuities, where the outer layer of the nuclear membrane merges with the inner one by means of a *U*-shaped bend (Figs. 9, 42). The width of these discontinuities usually measured between 300 and 1500 Å. The nucleus contains numerous maximally dense particles with a diameter of about 130 Å. They are usually clustered together in a marginal zone and

---

FIG. 11. (Left) Zymogen-cell mitochondrion (*M*). In the surrounding cytoplasm are part of a Golgi apparatus (*GA*), a vacuole-containing body (*VB*), and zymogen granules (*ZG*). Methacrylate embedded section fixed at pH 7.2. × 42,000.

FIG. 12. (Right) Zymogen-cell mitochondrion situated between long strands of α-cytomembranes. Vestopal embedded section fixed at pH 8.5. × 42,000.

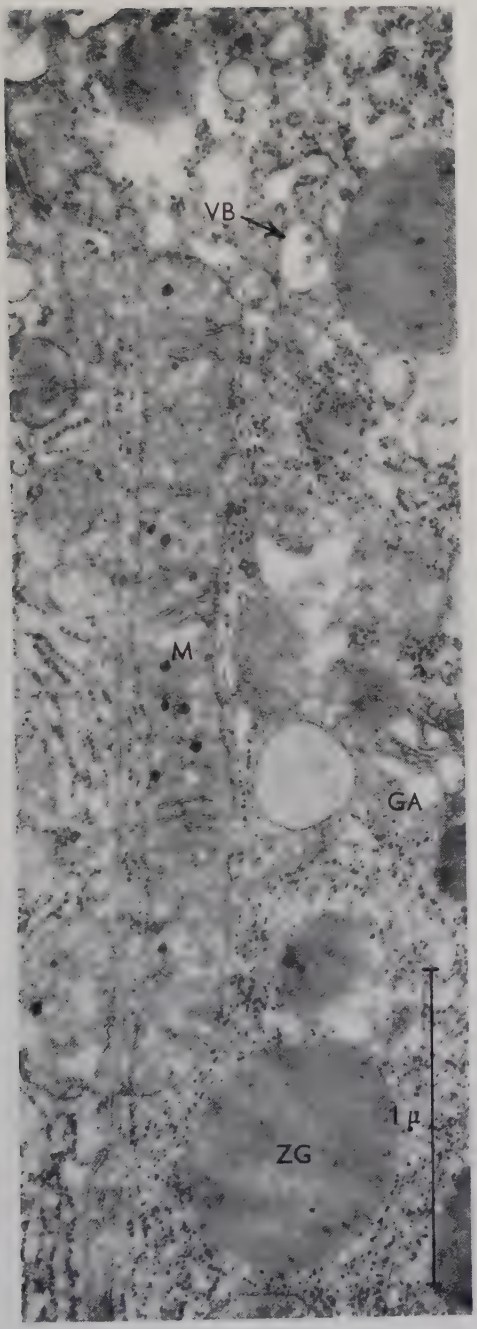



FIG. 11.

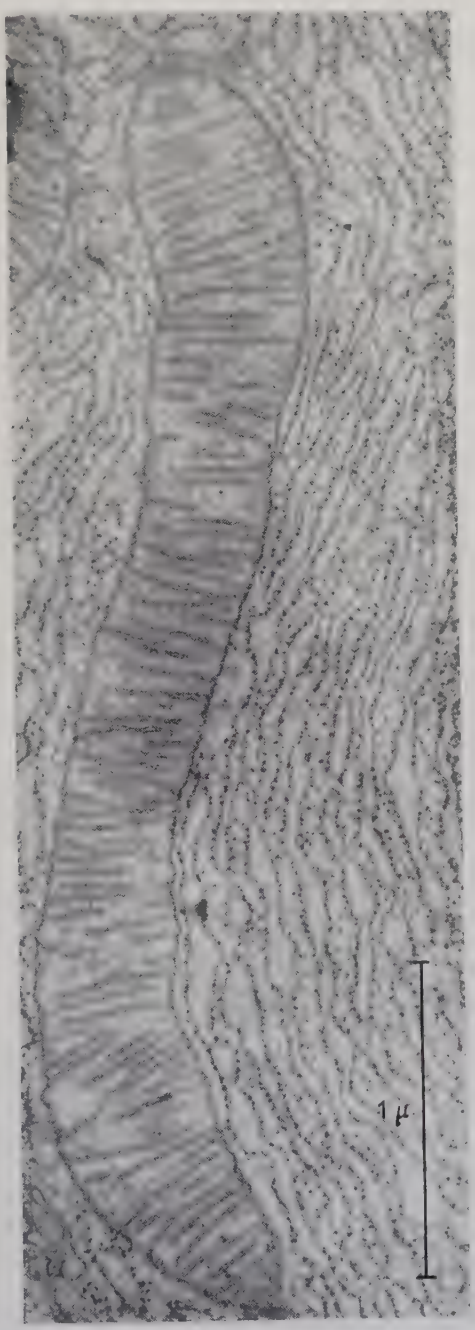



FIG. 12.

also in several indistinctly demarcated, more central regions. The number of marginal particles is reduced beneath the discontinuities in the nuclear surface membrane (Figs. 9, 42).

*Parietal cells*

Parietal cells occur along the entire length of the glandular tubule though only sporadically in the upper third of the gland. These cells border against the glandular lumen apically, against epithelial cells or intercellular lacunae (cf. p. 57) laterally, and against the basement membrane basally.

From the apical surface a number of *microvilli* project (Figs. 14–16). They have a diameter of about 730 Å and a maximum length of about 0.6 $\mu$. One observes in transversely sectioned microvilli a small number of dense spots which, though irregularly disposed, are on the whole confined to a narrow marginal belt (Fig. 44). The corresponding region of longitudinally sectioned microvilli contains dense filamentary structures the length of which often seems to approach that of the microvillus itself. Where the apical cell surface lacks such microvilli, its form is undulating, and in some places it demarcates irregularly shaped cytoplasmic bulges (Fig. 16). Often the apical *plasma membrane* which has a thickness of about 70 Å appears distinctly three-layered, a dense layer then being situated on either side of a less dense layer (Fig. 44). These layers are about equally thick.

A highly characteristic feature of the parietal cells are the *intracellular canaliculi* which sometimes are seen opening on the apical cell surface (Figs. 15, 16, 43). These canaliculi are very tortuous, and variously long portions of them are encountered throughout the cell. Profiles of one to three discrete intracellular canaliculi are seen in most sections through the central parts of a parietal cell (Figs. 13, 14). The canaliculi are approximately 1 $\mu$ in diameter. Sometimes pronounced canalicular dilatations may be observed, but almost without exception the cell then exhibits signs of poor preservation. The canalicular wall is continuous with the apical cell surface and carries microvilli in abundance. The plasma membrane and the microvilli of the canaliculi have the same appearance as the corresponding structures on the apical cell surface. However, some of the canalicular microvilli apparently are branched (Fig. 17).

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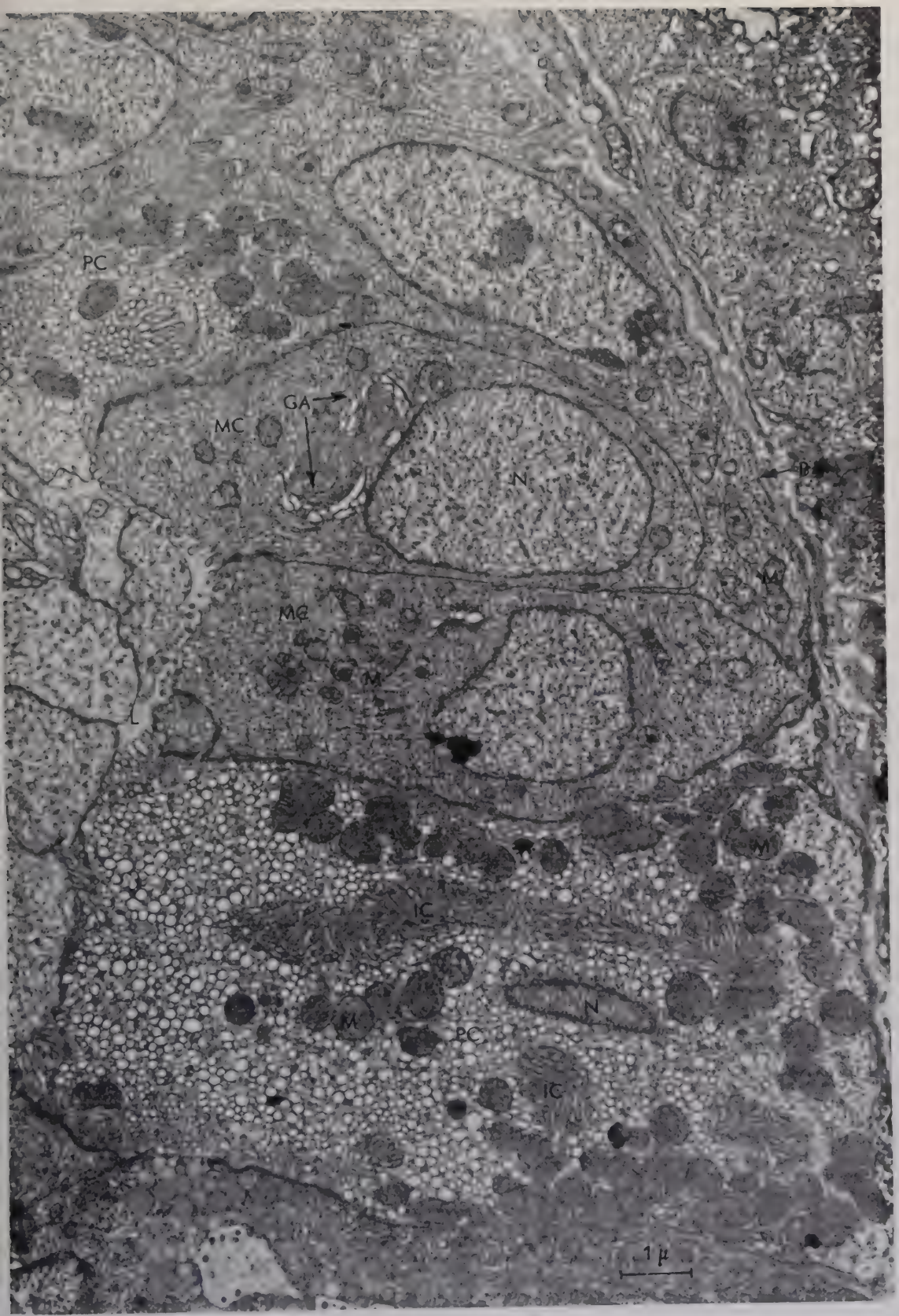
FIG. 13. Parietal cells (*PC*) and mucoid cells (*MC*). To the right is the basement membrane (*B*) and to the left the lumen (*L*) of the fundus gland, the latter being partly occluded by cytoplasmic projections from neighboring glandular cells. Intracellular canaliculi (*IC*), mitochondria (*M*) and numerous vacuoles are encountered in the parietal cells. In the mucoid cells, the most apical portions and the vicinity of the Golgi apparatus (*GA*) are the site of a small number of secretory granules. Some mucoid-cell mitochondria (*M*) contain dense granules. Several cell nuclei (*N*) are evident. Methacrylate embedded section fixed at pH 7.2, ×12,000.


PC
GA
MC
N
B
M
MC
M
L
M
IC
M
N
PC
IC
1 μ

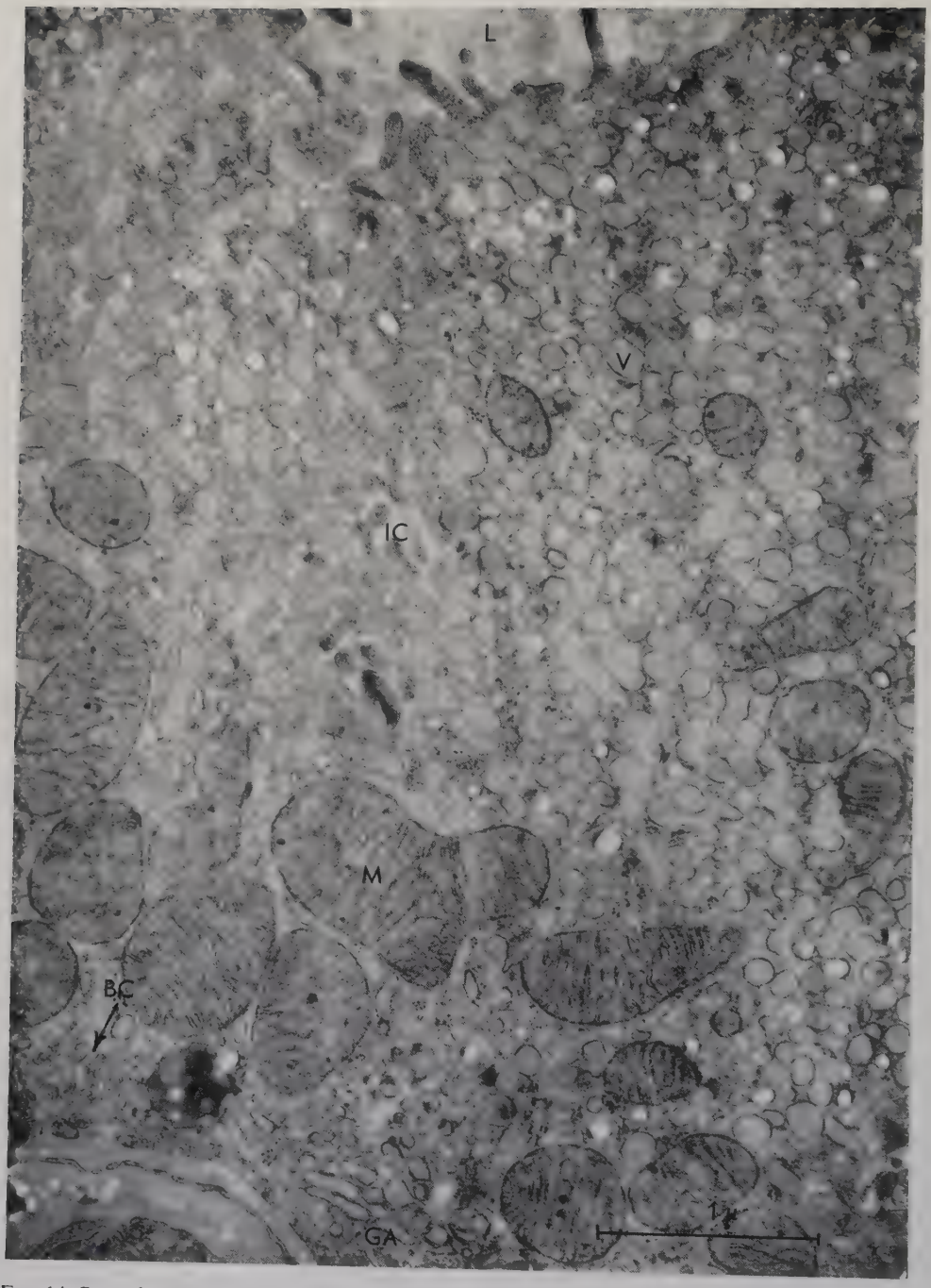



FIG. 14. Part of a parietal cell from which a number of microvilli project into the glandular lumen (*L*). The cytoplasm contains numerous vacuoles (*V*), some mitochondria (*M*), and a small Golgi apparatus (*GA*). A lipid droplet is seen left of the Golgi apparatus. The basal cell surface exhibits a concavity (*BC*) partly filled by cytoplasmic projections. Along the basal and apical cell surfaces and near the intracellular canaliculi (*IC*), the cytoplasm contains a much higher proportion of finely granular, moderately dense substance than elsewhere. It appears that the embedding medium is not present in some of the vacuoles. Vestopal embedded section fixed at pH 8.5. × 31,000.

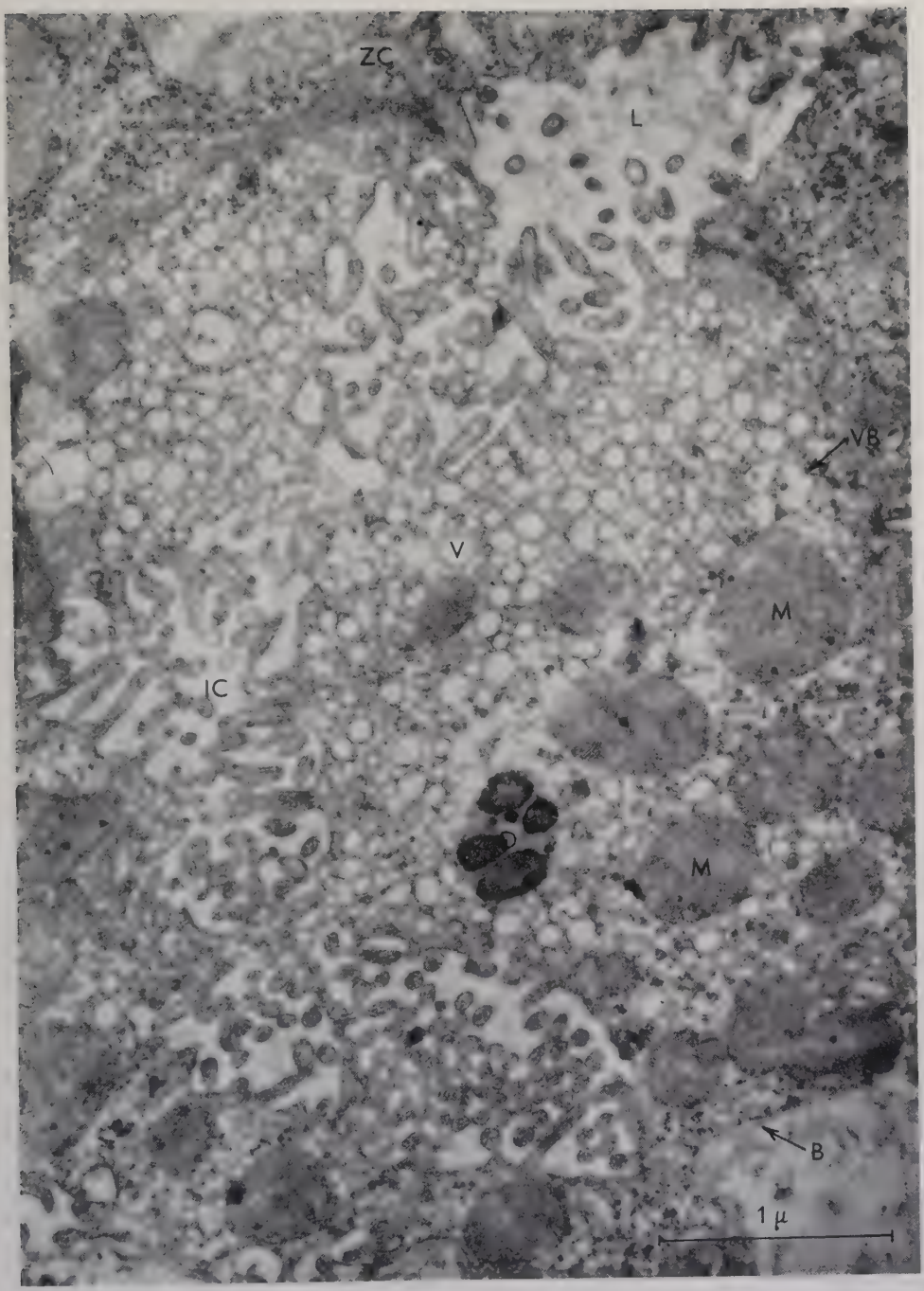



FIG. 15. Part of a parietal cell with an intracellular canaliculus (*IC*) extending from the basal portion of the cell (at the bottom of the figure) to the glandular lumen (*L*). Numerous microvilli containing a finely granular substance project from the canalicular walls. The cytoplasm contains mitochondria (*M*), vacuoles (*V*), vacuole-containing bodies (*VB*) and a dense microbody (*D*). Clusters of small particles occur throughout the cytoplasm but predominate basally. Below the basal cell surface lies the basement membrane (*B*). Part of a zymogen cell (*ZC*) appears in the upper left corner. Methacrylate embedded section fixed at pH 7.2. ×32,000.

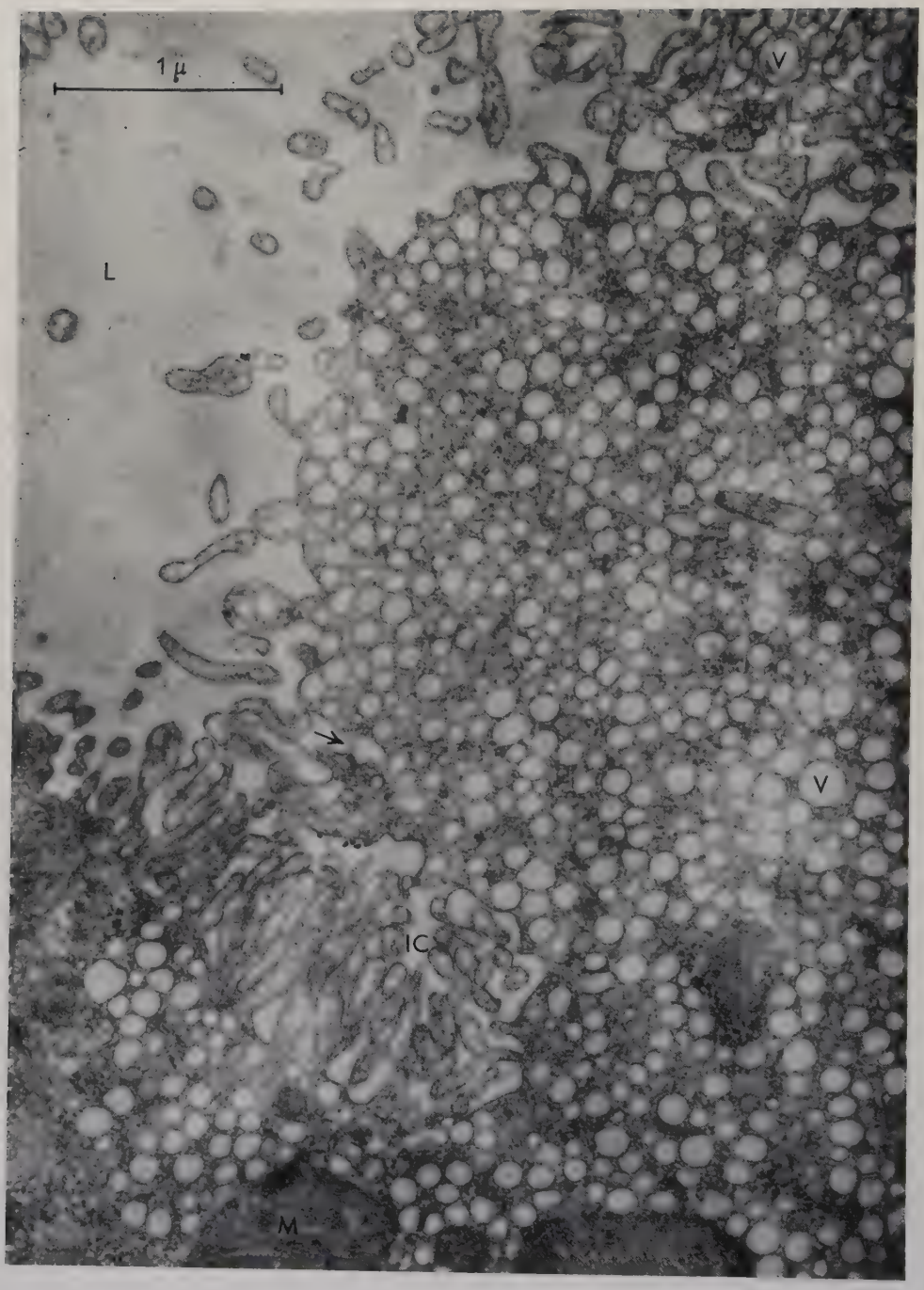



FIG. 16. Apical part of a parietal cell with an intracellular canaliculus (*IC*) opening into the glandular lumen (*L*). Microvilli abound in the canaliculus but are less numerous on the apical cell surface which also is provided with a few broader projections. The cytoplasm, occasionally even within the latter projections, contains a large number of vacuoles (*V*). At the arrow it appears that a vacuole communicates with the glandular lumen. Two mitochondria (*M*) are visible near the bottom of the figure. The cytoplasmic ground substance is considerably denser than after fixation at pH 7.2 Methacrylate embedded section fixed at pH 8.5. × 31,000.

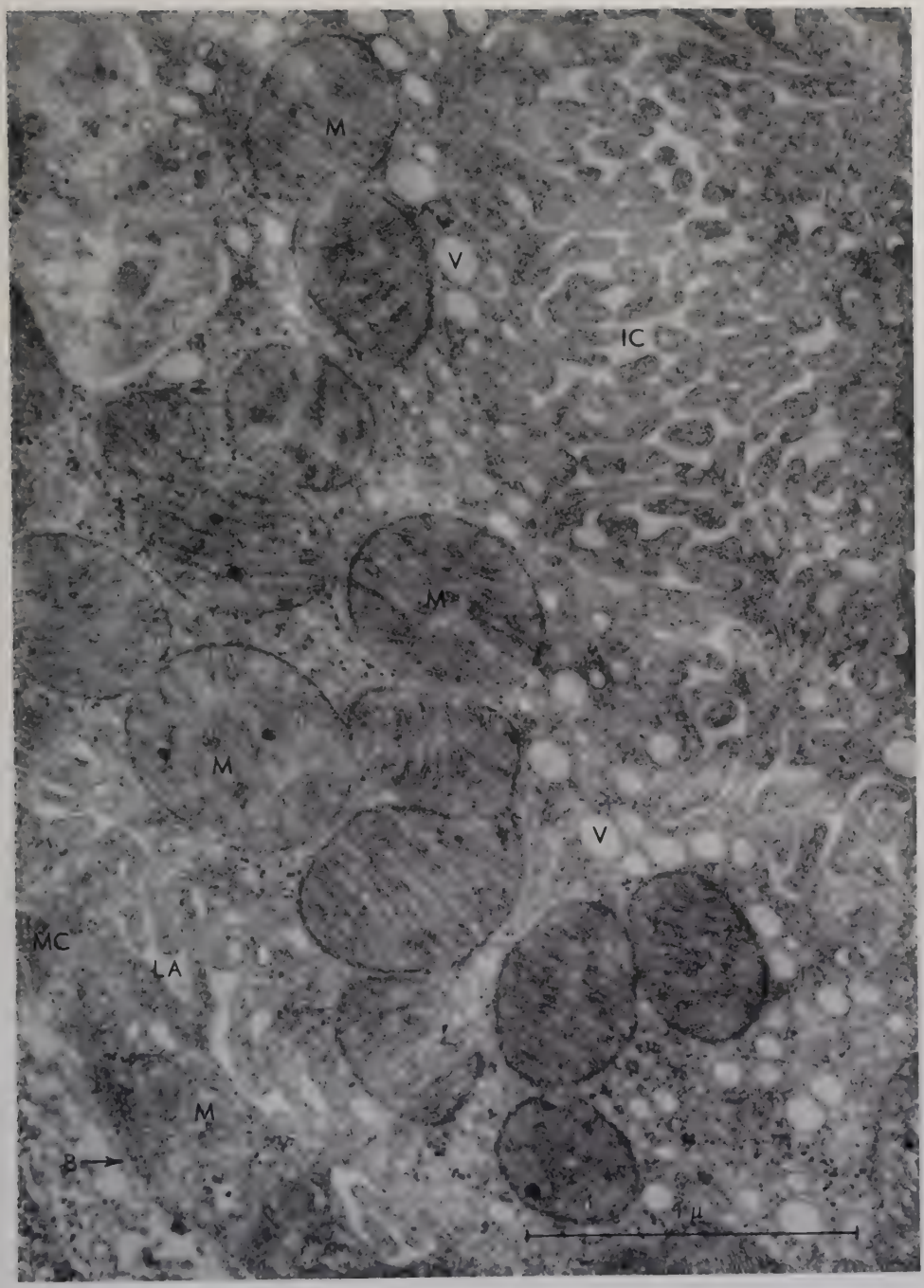



FIG. 17. Basal portion of a parietal cell. A number of microvilli which in some cases appear to branch project from the walls of the intracellular canaliculus (*IC*). The cytoplasm contains vacuoles (*V*) and mitochondria (*M*). The parietal cell borders against a mucoid cell (*MC*) in the lower left corner. The intercellular space here and there widens into small lacunae (*LA*) into which project cytoplasmic tongues. Standing on the basement membrane (*B*), the mucoid cell contains mitochondria (*M*) in which dense granules may be seen. The density of the ground cytoplasm in the two cells and of the mitochondrial matrix is comparatively high. Methacrylate embedded section fixed at pH 8.5. ×46,000.

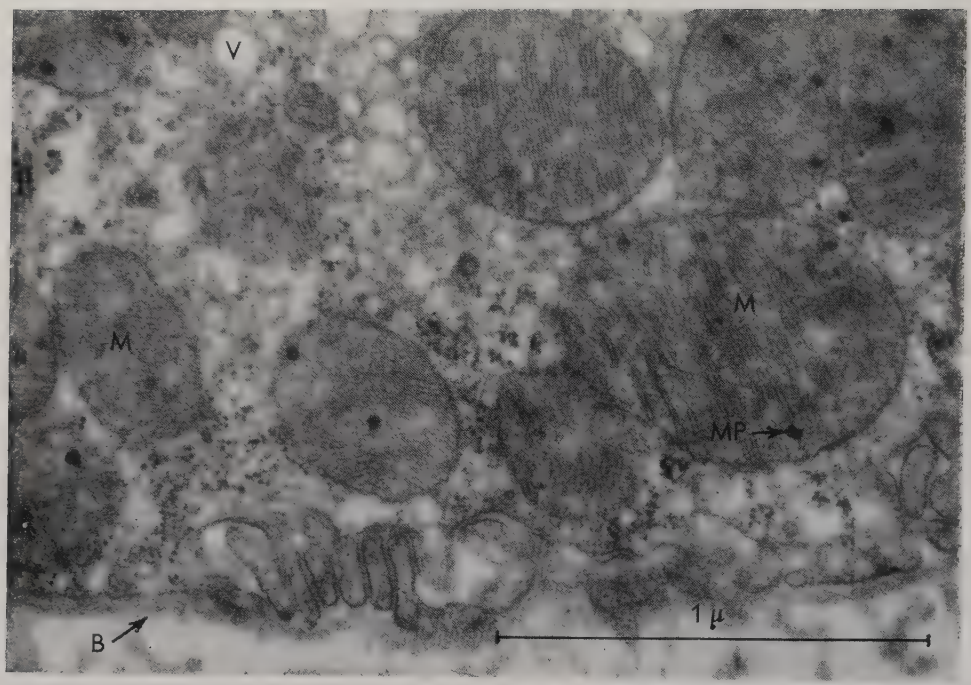



FIG. 18. Basal part of a parietal cell. The basal cell surface exhibits an irregularly formed concavity partly filled by cytoplasmic projections, arising from the bottom of the concavity, all these cell surface irregularities being bridged by the basement membrane (*B*). The cytoplasm contains mitochondria (*M*) with occasional mitochondrial particles (*MP*), a few vacuoles (*V*), and small particles. Methacrylate embedded section fixed at pH 7.2. × 56,000.

The lateral cell surfaces have *interdigitations* resembling those of the zymogen cells. In addition, from such surfaces which border against a particular type of mucoid cell (cf. pp. 56–57) longer cytoplasmic processes extend into the distended intercellular space (Fig. 17). Within the most apical portion of the lateral cell surfaces one regularly encounters a *terminal bar* of the same appearance as those in the zymogen cells (Fig. 23). The lateral plasma membranes are about 60 Å thick.

With few exceptions (see above) the *intercellular space* between a parietal cell and a neighboring epithelial cell has a fairly constant width of about 80 Å.

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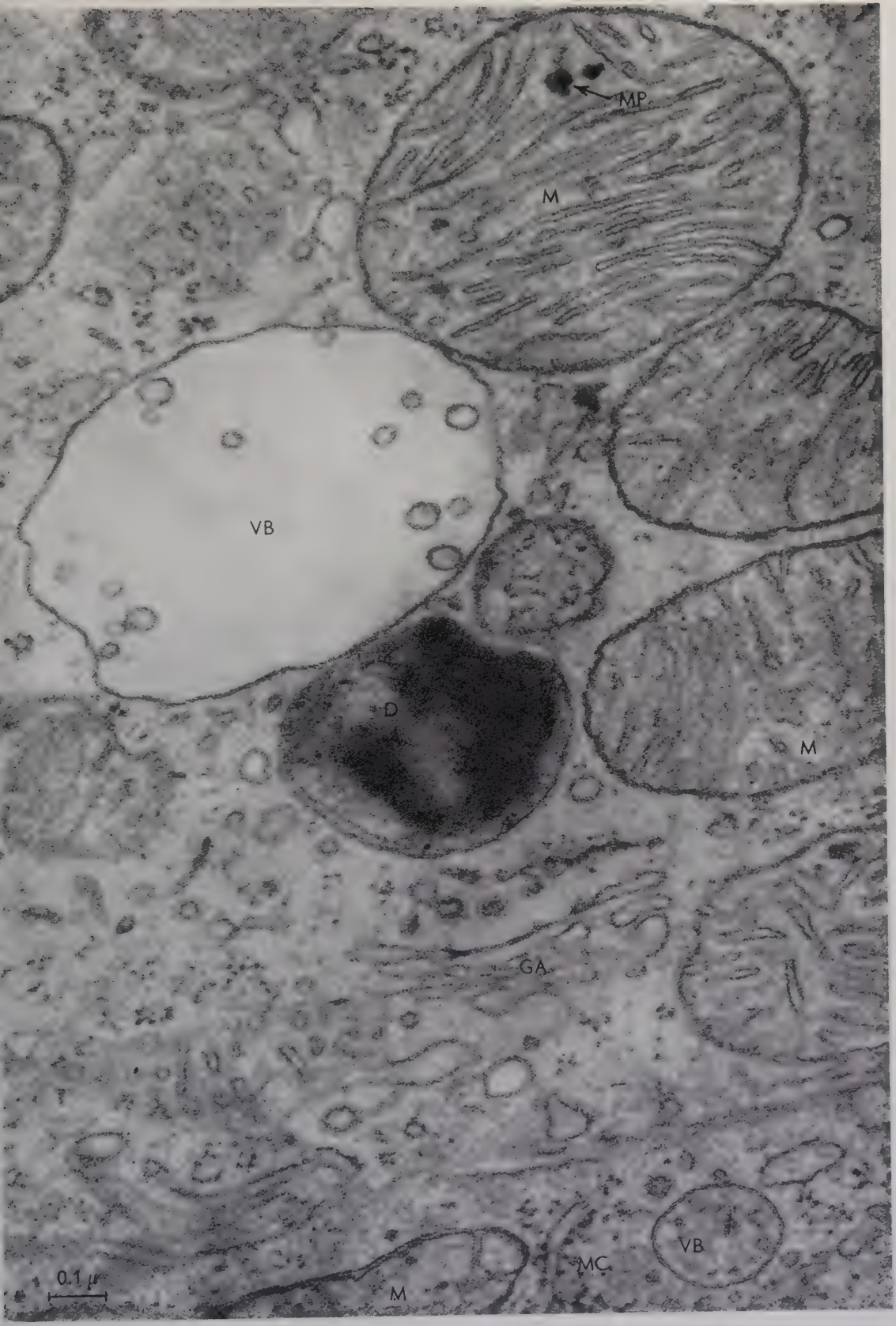
FIG. 19. Detail of a parietal cell, showing mitochondria (*M*), a small Golgi apparatus (*GA*), a vacuole-containing body (*VB*), and a dense microbody (*D*). The mitochondria contain inner membranes in abundance and a few mitochondrial particles (*MP*). In the lower part of the figure there appears a portion of a mucoid cell (*MC*), with a mitochondrion (*M*) and a vacuole-containing body (*VB*) in its cytoplasm. Vestopal embedded section fixed at pH 7.2. × 92,000.


MP
M
VB
D
M
GA
VB
MC
M
0.1 μ

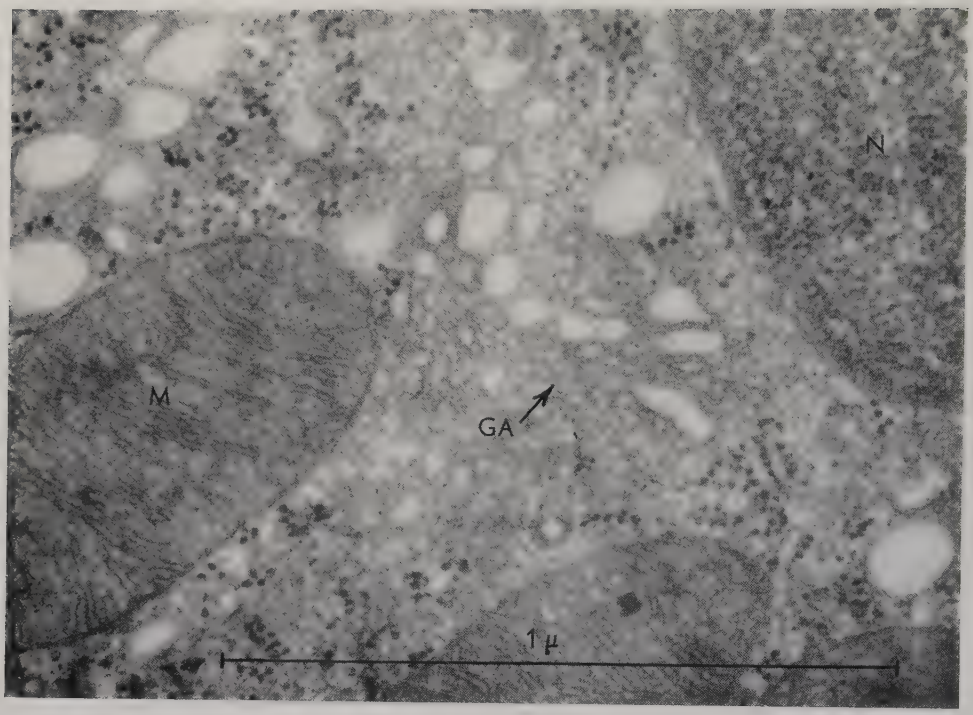



FIG. 20. A parietal-cell Golgi apparatus (*GA*). Being smaller than the Golgi apparatus in other fundus-gland cells, it comprises a few membranes, vacuoles and vesicles. The mitochondria (*M*) contain large numbers of inner membranes and occasional opaque particles. Part of the nucleus (*N*) is seen in the upper right corner. Methacrylate embedded section fixed at pH 8.5. × 88,000.

A number of shallow *concavities* are found on the basal cell surface (Figs. 14, 18). A few cytoplasmic processes resembling short microvilli arise from the bottom of these concavities and fill them more or less completely. In the majority of cases the basement membrane bridges these irregularities of the basal cell surface. The plasma membrane between these concavities is relatively straight.

*Vacuoles* occur in great numbers all over the parietal cell (Figs. 13–16). Occasionally they are even found in the cytoplasmic bulges projecting from the apical surface (Fig. 16). However, they are usually very sparse in a marginal zone some 1 or 2 $\mu$ wide next to the basal surface (Figs. 13–15, 18). The vacuoles are round or oval, have a diameter averaging about 0.14 $\mu$, and possess a smooth surface membrane some 70 Å thick which on electron micrographs of high resolution is seen to be composed of three layers (Figs. 22, 44). The vacuolar content is unstructured and of the same density as the embedding medium. In addition to these round or oval vacuoles, there

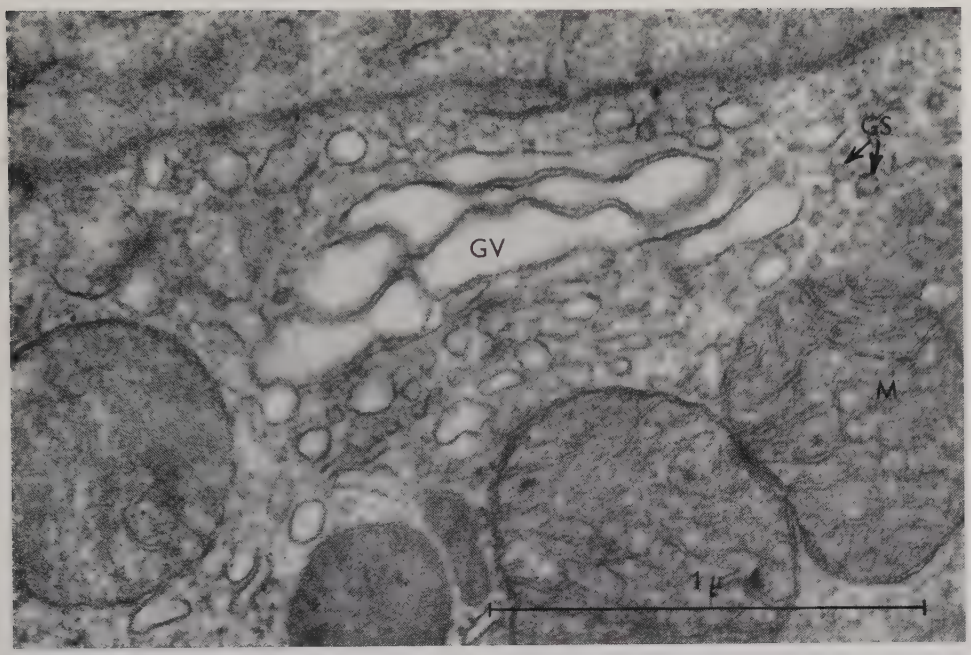



FIG. 21. A parietal-cell Golgi apparatus composed of membranes limiting irregularly shaped Golgi vacuoles (*GV*) and a few Golgi vesicles (*GS*). In the neighborhood of the Golgi apparatus there are a few mitochondria (*M*). Vestopal embedded section fixed at pH 8.5. × 58,000.

are a few tubular formations with similar contents and surface membrane. These tubules have a smaller diameter than the vacuoles.

At the base of occasional microvilli there are vacuolar invaginations of the cell surface which, however, generally are smaller than the cytoplasmic vacuoles (Fig. 16).

An abundance of *mitochondria* are scattered throughout the cytoplasm of the parietal cells but seem particularly numerous near the lateral and basal cell surfaces (Fig. 13). As seen in the sections the mitochondria are as a rule round or oval, the major axis seldom exceeding 2 $\mu$ and the minor axis averaging about 0.56 $\mu$. Along most of its course the mitochondrial outer membrane is visualized as a three-layered structure, with a dense layer about 30 Å thick on either side of a less dense layer about 50 Å thick. The inner membranes are on the whole parallel and disposed transversely, most of them extending from one side of the mitochondrion to the other (Figs. 14, 15, 17–20). Neighboring inner membranes occasionally form short anastomoses. The mean interspace between adjacent inner membranes is usually merely some 120 Å in the mitochondria of parietal cells and hence appreciably

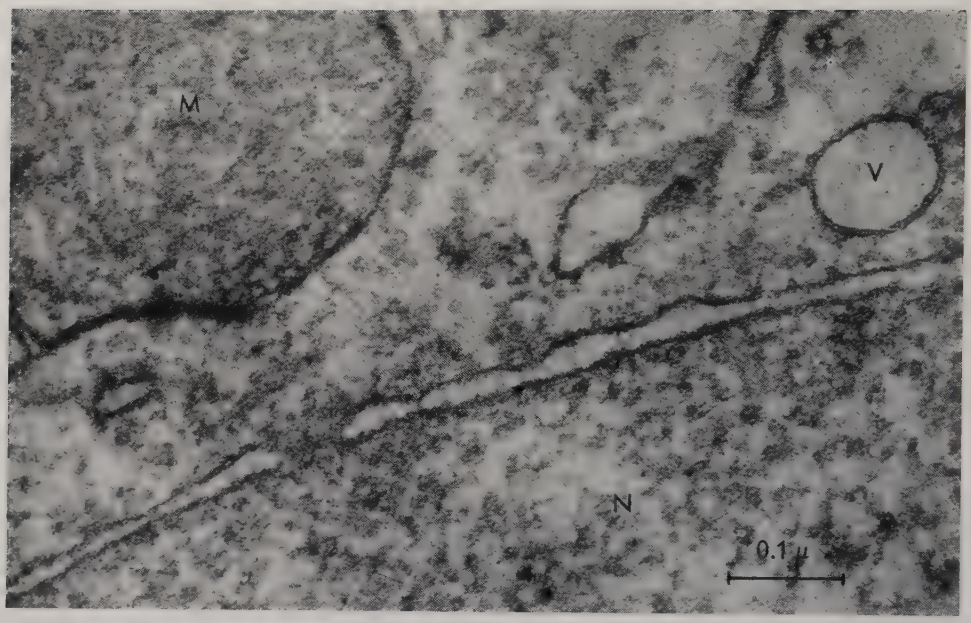



FIG. 22. Detail of a parietal cell. Part of the nucleus (*N*) appears in the lower-right corner. A discontinuity is visible in the three-layered nuclear membrane. A vacuole (*V*) having a three-layered surface membrane and a mitochondrion (*M*) are present in the cytoplasm. Vestopal embedded section fixed at pH 8.5. ×150,000.

smaller than in other epithelial cell types in the fundus gland. The inner membranes are composed of three layers, with a dense layer about 30 Å thick on either side or a less dense one about 70 Å thick. The less dense layer of some inner membranes is continuous with the less dense layer of the mitochondrial outer membrane. In addition the mitochondria contain occasional round, opaque particles with a diameter of about 360 Å (Figs. 18–20). These particles are unconnected with any membranes. The mitochondrial ground substance is moderately dense.

The organization of the *Golgi apparatus* is in principle the same as in the zymogen

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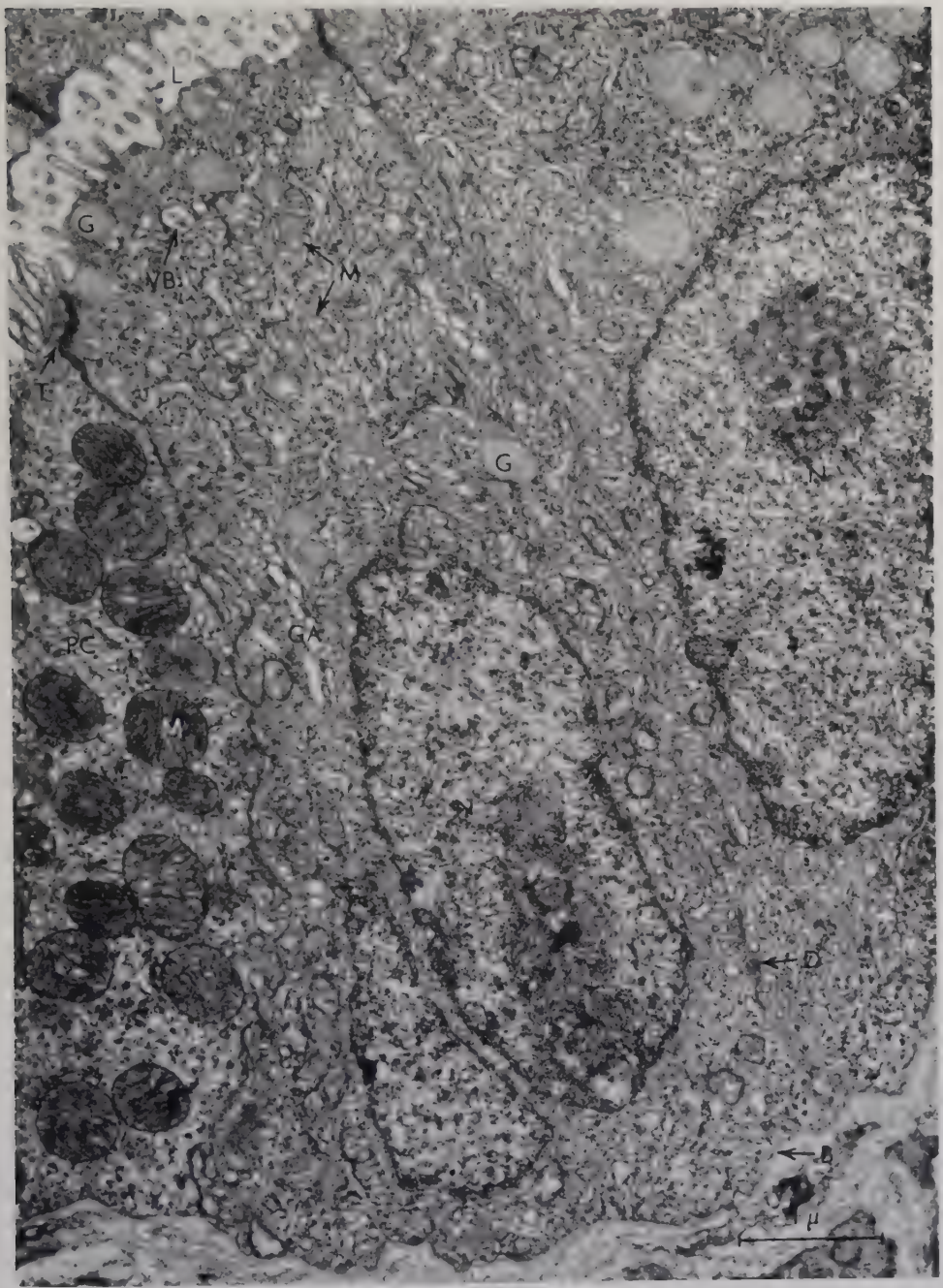
FIG. 23. Survey of a mucoid cell, including portions of neighboring mucoid and parietal (*PC*) cells. In the upper left corner is the glandular lumen (*L*) into which project a few microvilli. The cytoplasm contains but a few secretory granules (*G*), some lying in the immediate vicinity of the Golgi apparatus (*GA*). Mitochondria (*M*) in the mucoid cells are smaller than those in the parietal cell. The lateral plasma membrane has a terminal bar (*T*) most apically and, farther basally, it takes a more undulating course and occasionally exhibits a desmosome (*D*). A number of small, cytoplasmic vesicles may be observed near the lateral cell-surface irregularities. Beneath the slightly curved basal plasma membrane lies the basement membrane (*B*). *N*, nucleus, *VB*, vacuole-containing body. Methacrylate embedded section fixed at pH 8.5. ×20,000.

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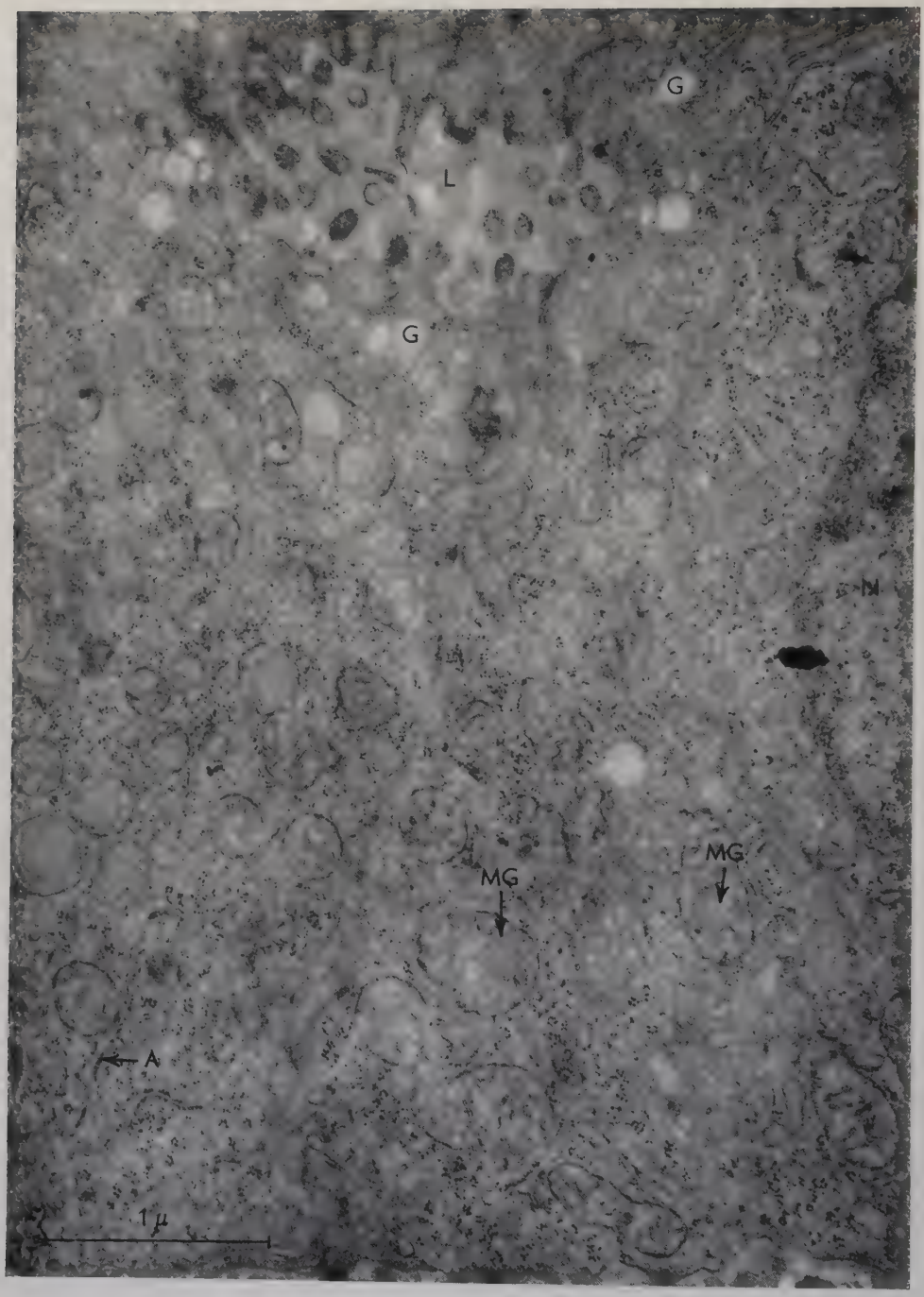


FIG. 24. Mucoid cells exhibiting rather few secretory granules (*G*), some mitochondria with intramitochondrial granules (*MG*), and a few α-cytomembrane strands (*A*). Near the top of the figure one finds the glandular lumen (*L*) into which project a few microvilli. Numerous small particles are dispersed throughout the cytoplasm. Part of a nucleus (*N*) is seen to the right. Vestopal embedded section fixed at pH 8.5. ×31,000.

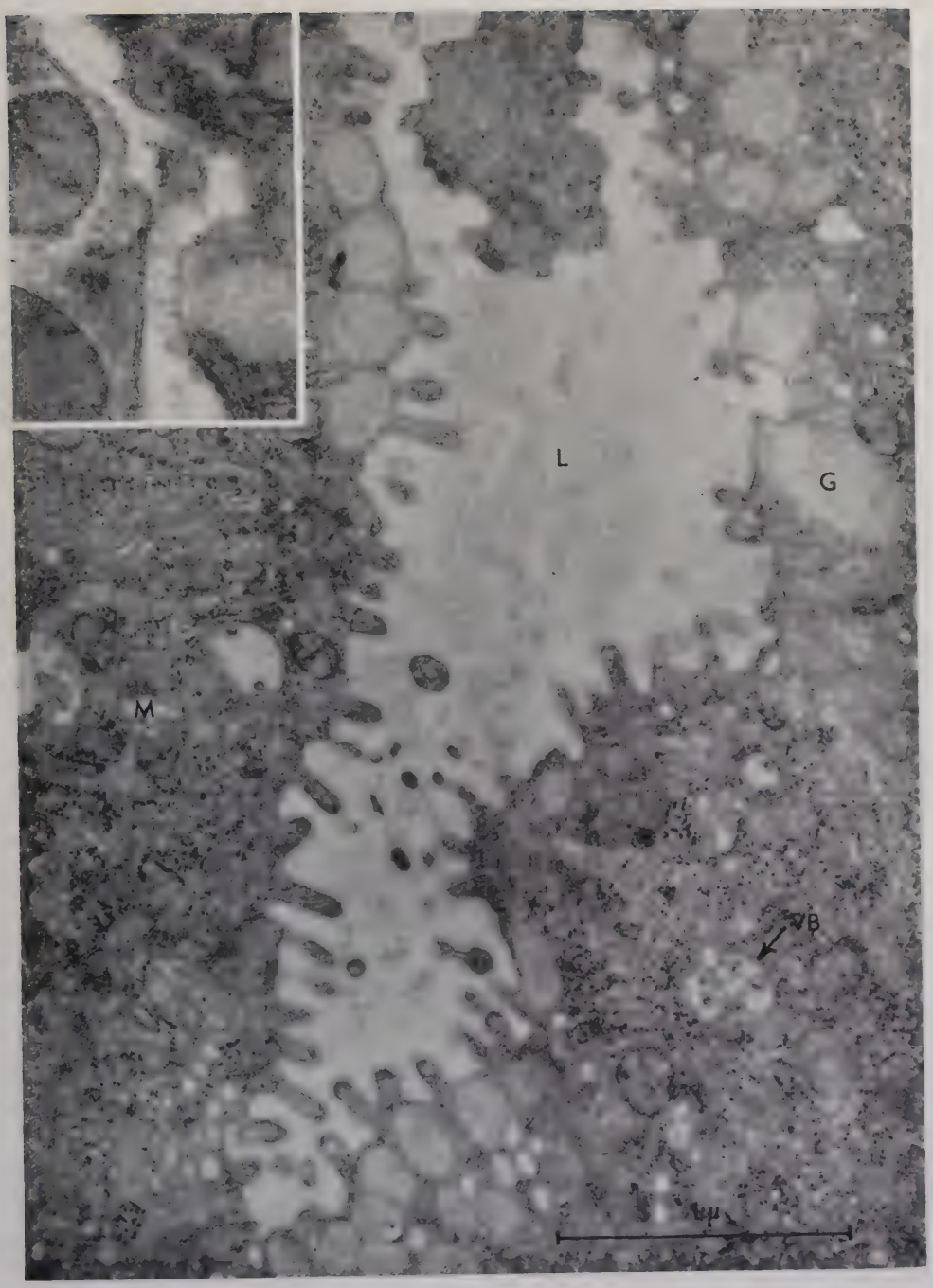


FIG. 25. A fundus-gland lumen (*L*) bounded by mucoid cells. Patches of a dense substance occur here and there in the lumen, especially close to the microvilli projecting into it. A more extensive region of dense material will be found nearer the top of the micrograph. The cytoplasm of the mucoid cells contains a few secretory granules (*G*), confined to the zone immediately beneath the apical plasma membrane, mitochondria (*M*), vacuole-containing bodies (*VB*), and numerous maximally dense particles. Methacrylate embedded section fixed at pH 8.5; 40,000. The inset depicts the three-layered apical plasma membrane of the mucoid cell. Methacrylate embedded section fixed at pH 7.2. ×67,000.

cells. Compared with the Golgi apparatus in this type of cell, however, that of the parietal cells is much smaller (Figs. 14, 19–21).

Round or polygonal, maximally dense *particles* with a mean diameter of about 120 Å occur in comparatively small numbers throughout the cytoplasm, usually in groups of three to eight, but sometimes singly and in contact with a pair of short membranes.

A section through the central parts of a parietal cell usually exhibits 2–4 *vacuole-containing bodies* (Figs. 15, 19). Encountered in all parts of the cytoplasm their shape is round or oval, but often they have a somewhat irregular outline. Their mean diameter is about 0.38 $\mu$, and they are bounded by a surface membrane approximately 70 Å thick. The inner substance—the density of which is roughly the same as that of the embedding medium—contains from 3 to 10, at times even more, vacuoles with a mean diameter of about 420 Å.

Sometimes *dense microbodies* (Figs. 15, 19) and *lipid droplets* (Fig. 14) are encountered in the cytoplasm of parietal cells. They are similar in appearance to the corresponding formations in the zymogen cells.

The density of the *ground cytoplasm* is low, except marginally along the apical and basal cell surfaces and near the intracellular canaliculi. In all the latter regions the cytoplasm is finely granular and moderately dense.

The round or oval *nucleus* is found in the basal half of the cell. Ultrastructurally it is similar to the nucleus in zymogen cells apart from the fact that it appears to have fewer maximally dense particles on its surface membrane. No deep indentations of the nuclear outline were encountered, and only occasionally were discontinuities observed in the nuclear membrane (Fig. 22).

### *Mucoid cells and argyrophil cells*

To a very large extent mucoid cells and argyrophil cells fixed at pH 7.2 and embedded in methacrylate proved to be poorly preserved. In so far as adequate preservation was obtained at this pH, the observed appearances were compatible with those seen in corresponding methacrylate-embedded cells fixed at pH 8.5.

## Methacrylate Embedded Tissue Fixed at pH 8.5

The ultrastructure of methacrylate embedded fundus glands fixed at pH 8.5 deviated in no major respect from that observed in similarly embedded, well-preserved specimens fixed at pH 7.2. Notably, however, the proportion of well-preserved cells was appreciably higher than after fixation at pH 7.2.

Only those formations which differed in structure and/or dimensions from corresponding formations described in specimens fixed at pH 7.2 will be considered.

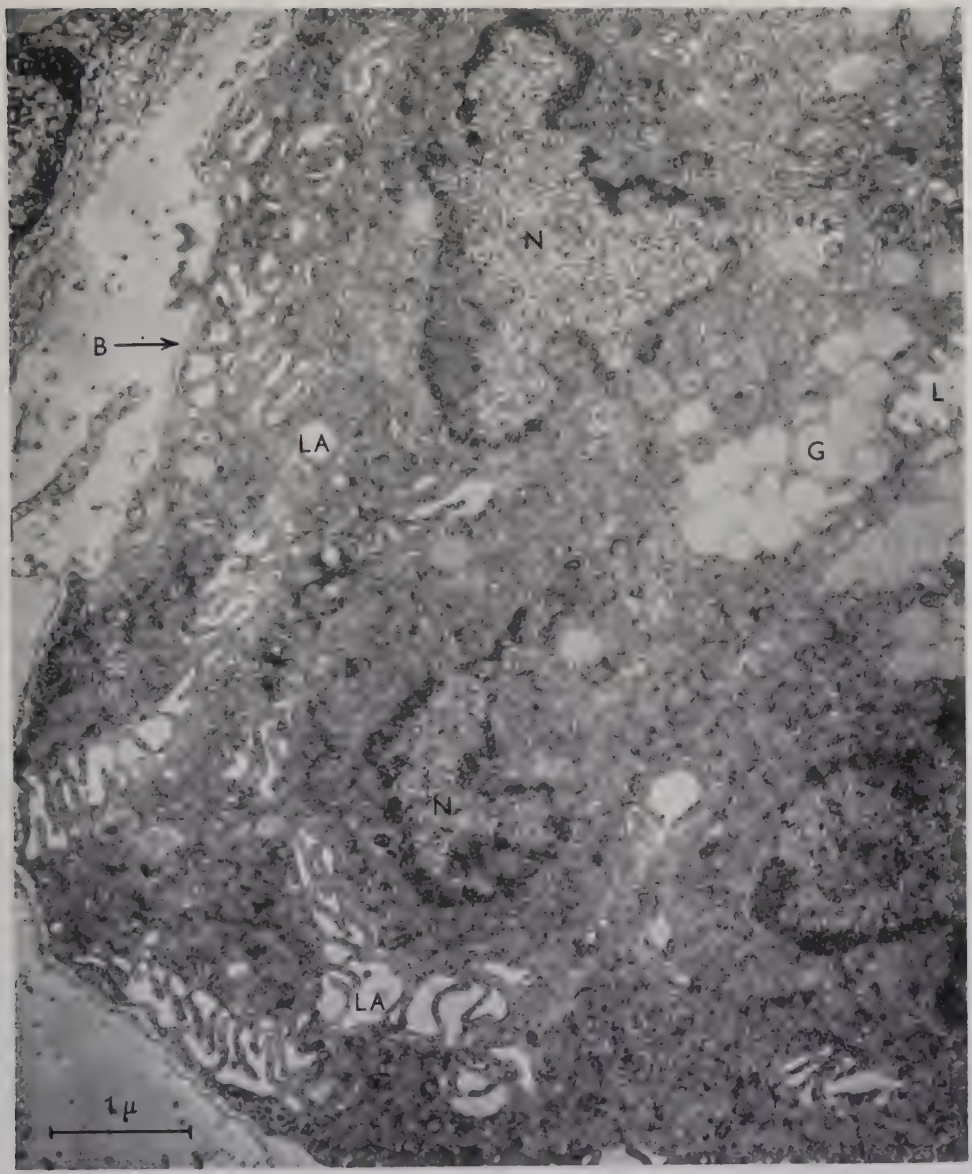


FIG. 26. A portion of a fundus gland composed of mucoid cells exclusively. The cytoplasm beneath the glandular lumen (*L*) contains a number of secretory granules (*G*). To the left are the basal cell surfaces, together with the accompanying basement membrane (*B*). Widenings of the intercellular space between the basal parts of adjacent cells form lacunae (*LA*) into which project long cytoplasmic tongues. The irregularly outlined nuclei (*N*) seem comparatively small and are rather dense. So is the cytoplasmic ground substance, making it difficult to discern the cytoplasmic organelles. Methacrylate embedded section fixed at pH 8.5. ×18,000.

*Zymogen cells*

Though the shape of the *zymogen granules* remains unchanged, these are, with occasional exceptions, less dense than at pH 7.2 (Fig. 7). Some zymogen granules have a dense segment. On the whole, however, all zymogen granules in the same cell are equally dense. The surface membrane of the zymogen granule is not seldom incomplete. The mean diameter of the zymogen granules does not differ significantly from that at pH 7.2.

The density of the cytoplasmic *ground substance* is markedly higher than at pH 7.2 which, particularly at low magnifications, makes it difficult to distinguish details of individual cells (Fig. 7). The *nuclear* ground substance also exhibits a higher density than at pH 7.2.

*Parietal cells*

The *microvilli* on the apical cell surface and in the intracellular canaliculi have an average diameter of some 810 Å, a figure which is statistically significantly higher than the value of 730 Å obtained for pH 7.2.

Apart from the higher electron density of their ground substance, the *mitochondria* have on the whole the same appearance as at pH 7.2 (Figs. 17, 20). The length of the minor axis averages 0.47 $\mu$ at pH 8.5 and is not significantly different from that at pH 7.2.

The *ground cytoplasm* is homogeneous in appearance and appreciably denser than after fixation at pH 7.2 (Figs. 16, 17, 20). The density of the *nuclear* substance is also increased.

*Mucoid cells*

Fixation of the gastric mucosa at pH 8.5 greatly improves the preservation of the mucoid cells and consequently facilitates their electron microscopic examination. Although the sections include some imperfectly preserved mucoid cells also after fixation at this pH level, the majority of these cells exhibited no signs of poor preservation.

The mucoid cells are found on the surface of the gastric mucosa and in the upper part of the fundus glands. Indeed, this portion of the glandular tubule may occasionally be composed exclusively of mucoid cells (Fig. 1). Their apical surface

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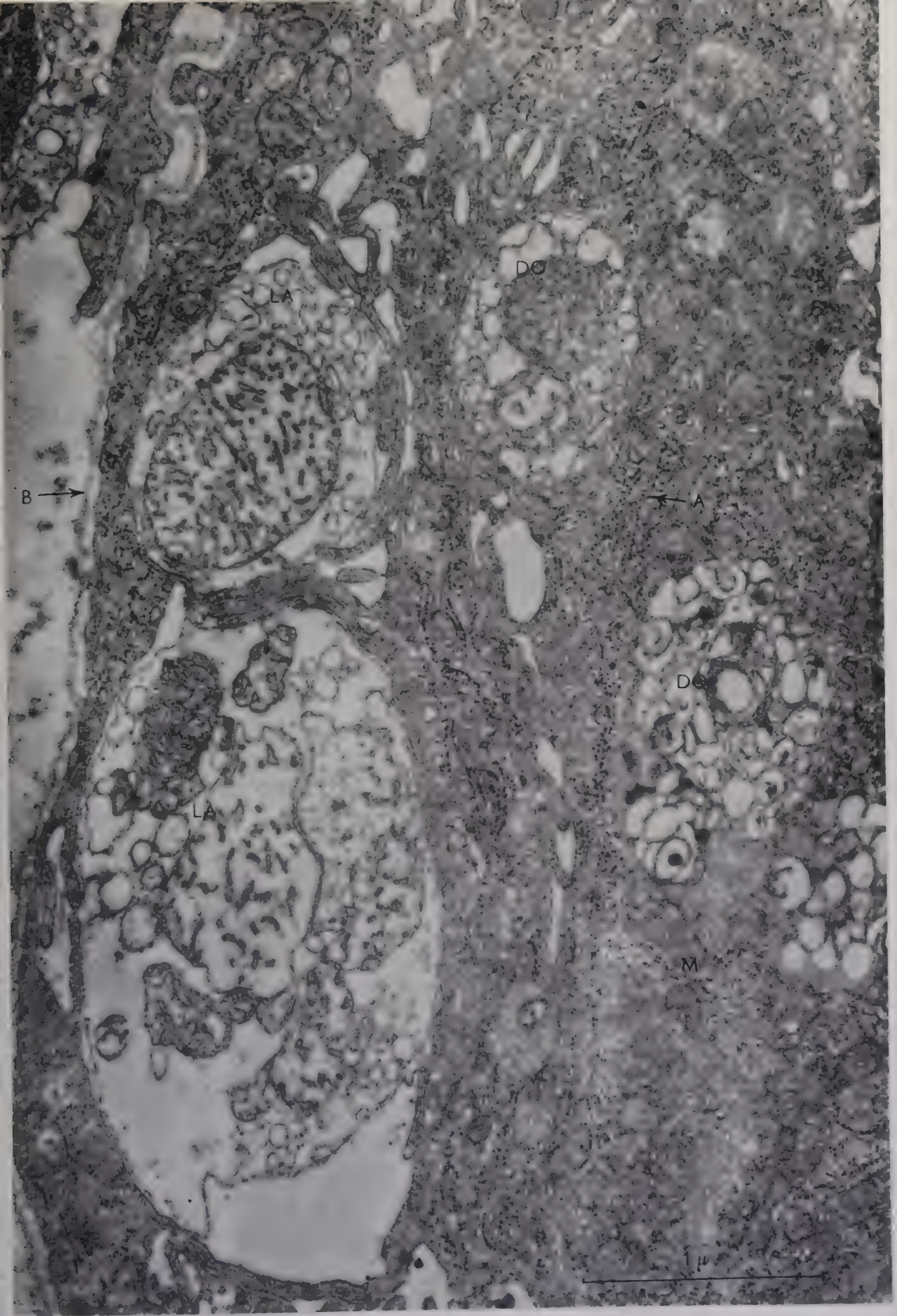
FIG. 27. Basal parts of mucoid cells with wide intercellular lacunae (*LA*) containing remnants of markedly altered organelles. Only some are barely identifiable, as mitochondria. Similar regions on the right, apparently within the cytoplasm of a mucoid cell, also seem to contain disintegrating cellular organelles (*DO*). The cytoplasmic ground substance is rather dense. The basal plasma membrane and the basement membrane (*B*) are situated to the left. *M*, mitochondrion; *A*, $\alpha$-cytomembrane. Methacrylate embedded section fixed at pH 8.5. $\times$40,000.


LA
DC
B
A
DC
LA
M
1 μ

borders against the glandular lumen, their lateral surfaces against epithelial cells or intercellular lacunae (see below), and their basal surface against the basement membrane.

The apical *plasma membrane*—which is about 70 Å thick—demarcates a small number of short, stubby projections and occasional more slender microvilli (Figs. 13, 23–25, 29). This part of the plasma membrane may sometimes be seen to be composed of two dense layers with an intervening less dense layer (Fig. 25) all three layers having approximately the same thickness.

Being about 50 Å thick, the lateral plasma membranes have an essentially straight course in their most apical regions (Figs. 23, 25). Here a *terminal bar* is regularly encountered which is of about the same appearance and dimensions as in the zymogen cells (Fig. 23). *Desmosomes* similar to those of zymogen cells are observed at all levels of the lateral surfaces facing adjacent mucoid cells (Fig. 23). As a rule the plasma membrane basal to the terminal bar is more undulated, and usually it becomes increasingly so on approaching the basal cell surface (Fig. 23). This gives rise to the formation in this region of a more or less complex pattern of broader and narrower cytoplasmic projections. Not seldom one observes at the base of these projections small invaginations of the plasma membrane, beneath which a row of small vacuoles may often be present in the cytoplasm (Figs. 23, 28).

The basal portion, finally, of the plasma membrane is fairly undulated (Figs. 23, 24, 26, 27).

The configuration and size of the *intercellular space* between two adjacent mucoid cells are very variable. Straight plasma membrane portions are usually a fairly constant distance of some 80 Å apart. Where the plasma membranes have a more undulated course, the appearance and dimensions of the intercellular space may vary a great deal, depending upon the degree of interdigitation of the cytoplasmic projections of adjacent cells. One may encounter anything from minute often oval dilatations to highly irregular lacunae measuring several $\mu$ across (Figs. 26, 27). Whilst the intercellular space practically always contains a homogeneous substance of the same density as the embedding medium, it may occasionally be finely floccular and more dense. Membranous formations have been observed in some of the larger lacunae. These formations sometimes look like swollen or otherwise altered mitochondria, vacuoles or other common cell organelles (Fig. 27). Many of them are so altered in one way or another, however, that identification is not feasible. Those parts of the intercellular lacunae which contain such formations are demarcated by a membrane of the same order of thickness as the lateral plasma membranes of mucoid cells (Fig. 27). Just before the points where the lateral cell surfaces merge with the basal cell surface, the intercellular space as a rule returns to a width of some 80 Å (Figs. 26, 27).

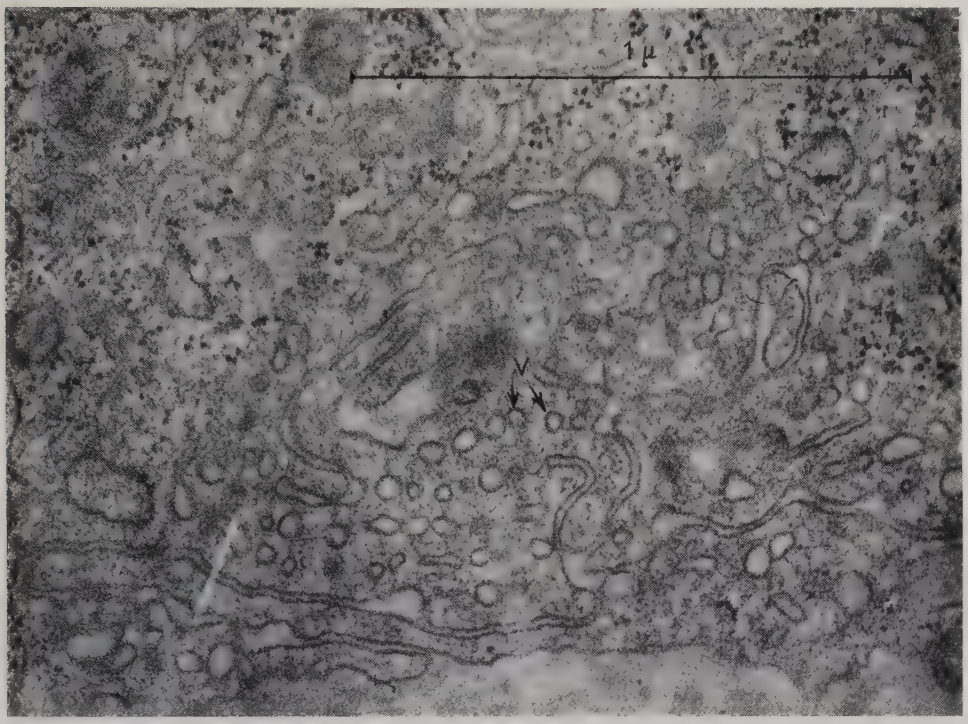



FIG. 28. Basal portions of mucoid cells with very tortuous plasma membranes on their lateral surfaces. The vacuoles (V) appearing in rows in the cytoplasm seem to have been pinched off from invaginations on the lateral cell surfaces. Vestopal embedded section fixed at pH 8.5. ×73,000.

The cytoplasm of cells bordering against conspicuous lacunae in the intercellular space usually is so very dense that the intracellular organelles are hard to distinguish (Figs. 26, 27). The nucleus in such cells has a very indented margin and usually contains a substance which is more dense than the nuclear substance of other mucoid cells (Fig. 26). Moreover, there are in many such cells a few indistinctly outlined, round or oval areas whose diameter often exceeds 1 $\mu$. Within these areas swollen, apparently disintegrating mitochondria and other cellular organelles can be identified (Fig. 27).

Similar but less pronounced irregularities of the intercellular space may be found between mucoid cells and other types of epithelial cell (Fig. 17).

The number and mode of distribution of the *secretory granules* vary greatly from one mucoid cell to another. They are encountered throughout the cytoplasm but are found predominantly in a marginal zone along the apical cell surface. Although this cytoplasmic zone can be up to several $\mu$ wide (Fig. 29), it may just as often be

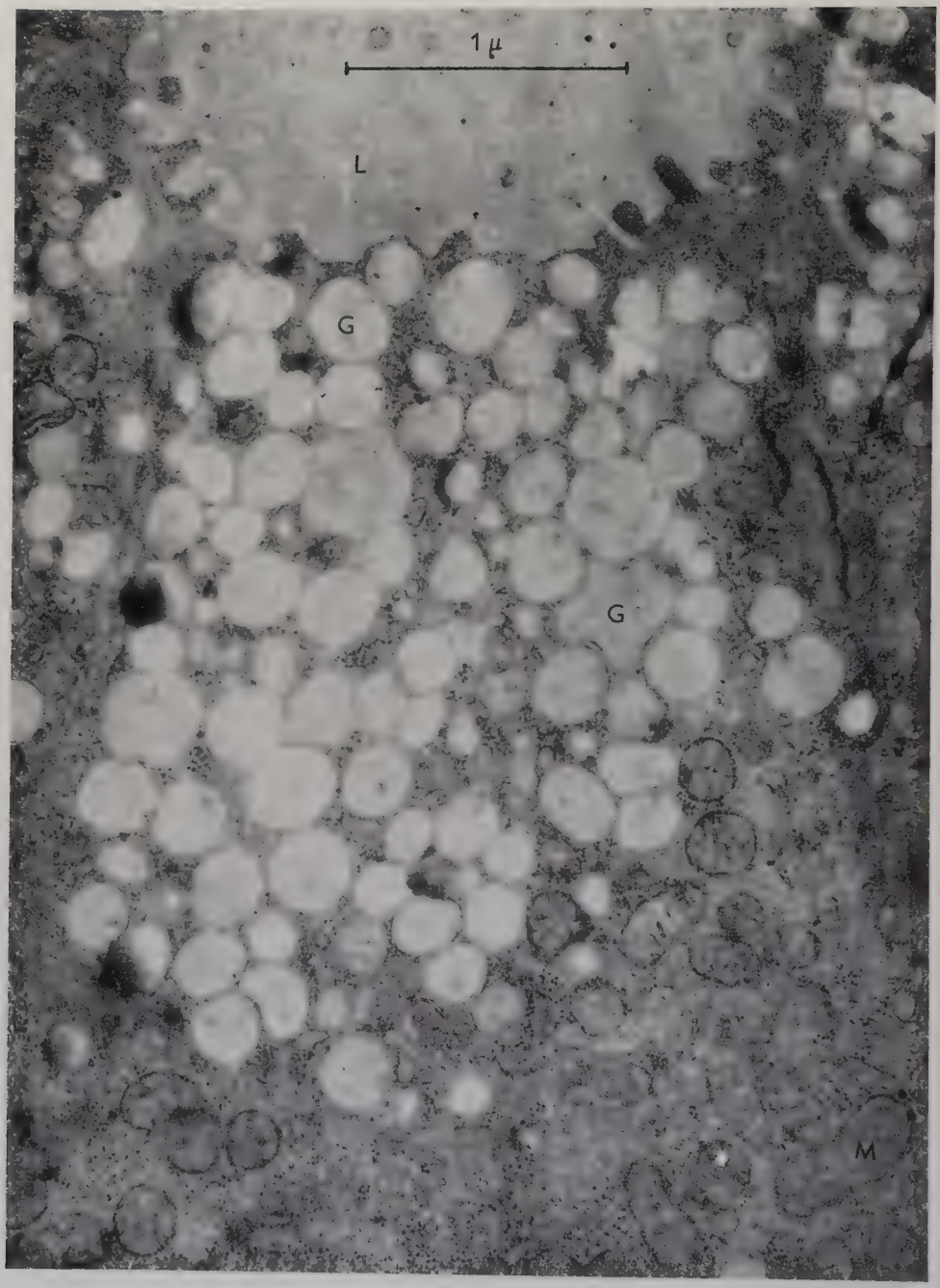



FIG. 29. Apical portion of a mucoid cell. It contains a large number of secretory granules (*G*) which sometimes seem to compress one another. Some of them have a narrow, moderately dense zone just beneath the surface membrane. Some mitochondria (*M*) and numerous dense particles are present in the cytoplasm. A small number of stubby microvilli project into the glandular lumen (*L*). Vestopal embedded section fixed at pH 8.5. ×38,000.

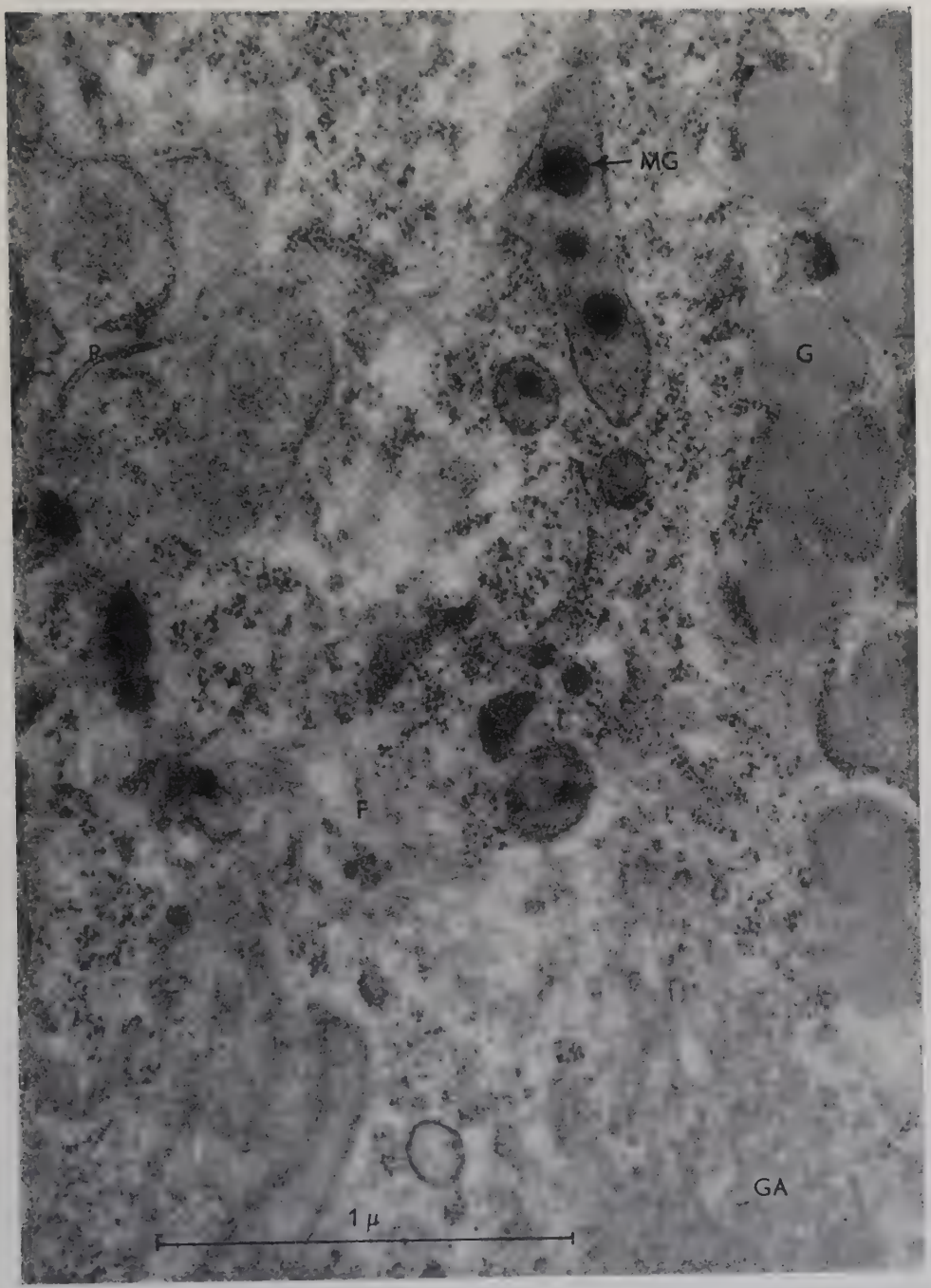
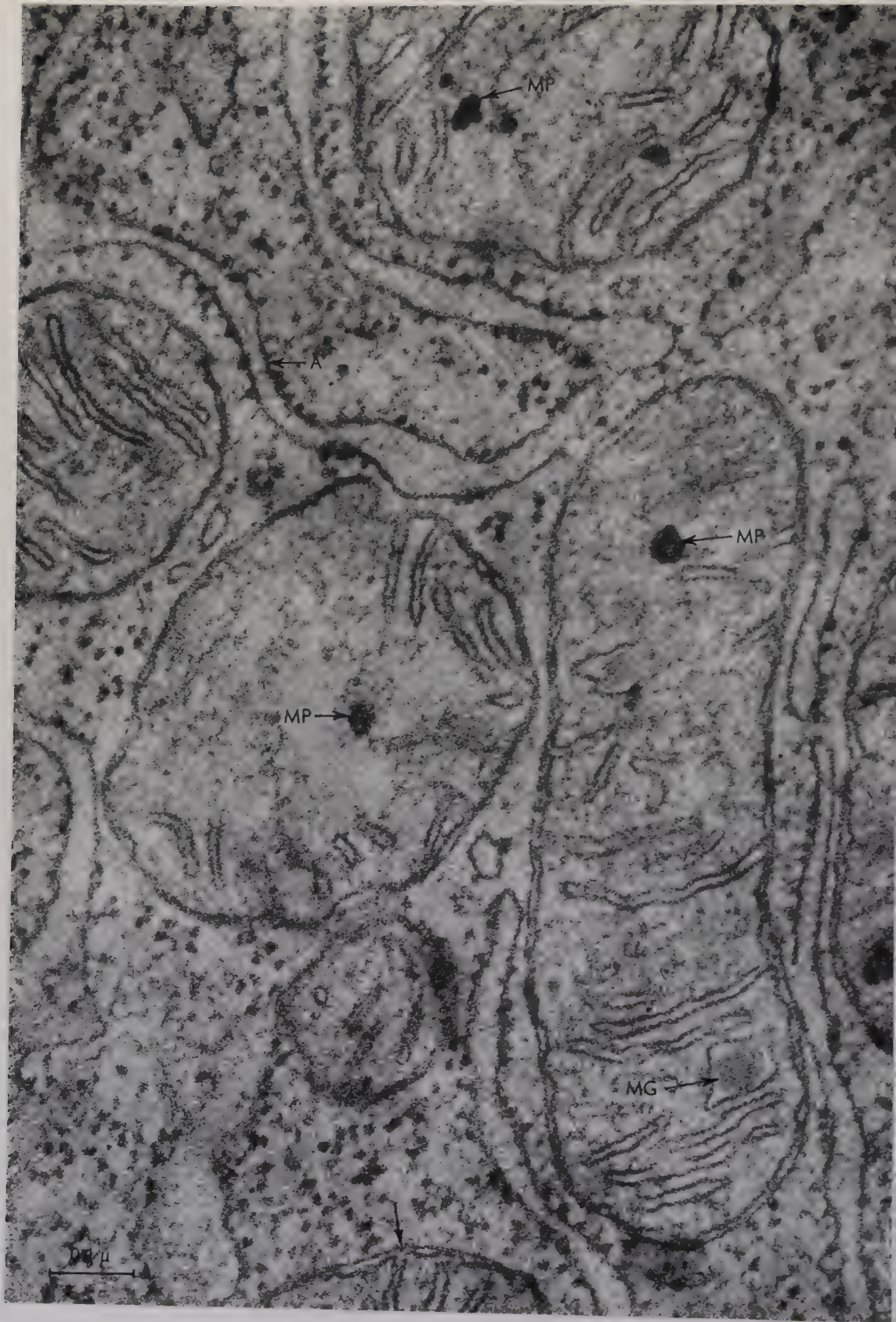



FIG. 30. Parts of mucoid cells. The mitochondria include membrane-bound, dense granules (*MG*). The cytoplasm contains secretory granules (*G*), part of a Golgi apparatus (*GA*) and some thin fibrillar structures (*F*). The lateral plasma membrane (*P*) appears to the left. Methacrylate embedded section fixed at pH 8.5. 57,000.


MP
A
MP
MP
MG
0.1 μ

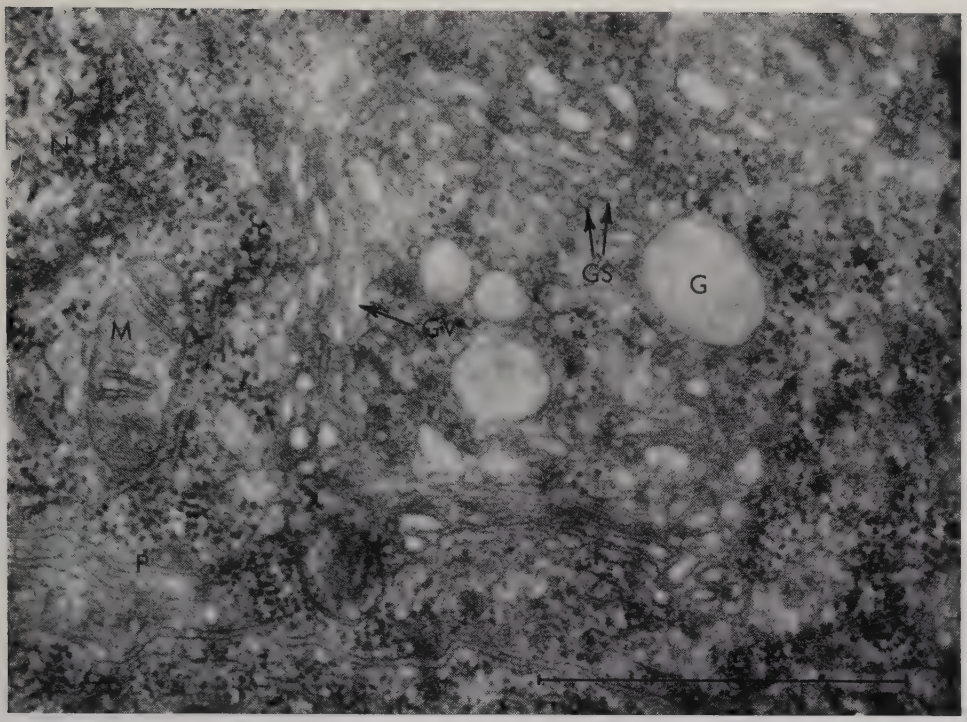



FIG. 32. A mucoid-cell Golgi apparatus comprising membranes, flat vacuoles (*GV*), and numerous vesicles (*GS*). In the neighborhood there are a few (newly created?) secretory granules (*G*) of varying size. A mitochondrion (*M*) and a segment of the nucleus (*N*) are situated to the left, and a piece of the highly undulated lateral plasma membrane (*P*) appears near the bottom. Methacrylate embedded section fixed at pH 8.5. ×48,000.

merely the width of a single, occasionally incomplete row of granules (Figs. 13, 23–25). Usually, however, it falls somewhere between these two extremes.

These secretory granules have a round or oval shape with a mean diameter of approximately 0.33 $\mu$. Deviations from these basic forms occur in cells where secretory granules are very abundant and lie so closely packed that neighboring granules seem to compress one another (Figs. 29, 30). Many of the secretory granules are observed to possess a distinct surface membrane. Most secretory granules have a homogeneous

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FIG. 31. View of mucoid-cell mitochondria and surrounding cytoplasm. Notable features are a mitochondrial granule (*MG*) surrounded by a loosely fitting membrane and also a number of opaque mitochondrial particles (*MP*) which apparently are unconnected with any membrane. The arrow indicates a point of contact between the less dense layers of the outer and an inner mitochondrial membrane. Some $\alpha$-cytomembranes (*A*) are seen. Vestopal embedded section fixed at pH 7.2. ×140,000.

content of moderate to high electron density, the density of granules within the same cell being approximately the same.

The majority of the *mitochondria* in the mucoid cell are encountered in the supranuclear and paranuclear regions of the cytoplasm (Figs. 13, 23). Their shape is usually round or oval but may be elongated. The mean minor diameter is about 0.16 $\mu$ and the length, as it appears in the sections, seldom exceeds 2 $\mu$. Along most of the mitochondrial perimeter the outer membrane appears as a three-layered structure comprising a dense layer some 30 Å thick on either side of a less dense layer about 40 Å thick (Fig. 31). The comparatively few inner membranes are usually transversally disposed. They are also composed of three layers, two dense ones being about 30 Å thick and a less dense intermediate one some 70 Å thick. Occasionally the less dense layers of the mitochondrial outer and inner membranes appear to be fused (Fig. 31).

Moreover, many of the mitochondria in the mucoid cells contain one or two round, homogeneous granules having a mean diameter of about 0.11 $\mu$ (Figs. 1, 24, 30, 31, 34). Their electron density varies but is often great. Most of them have a loosely fitting membrane of approximately the same thickness as the dense layers of the mitochondrial inner membranes. The moderately dense mitochondrial ground substance also contains occasional other, irregularly outlined, opaque particles unconnected with any membrane (Fig. 31). The diameter of these latter particles averages about 340 Å.

In principle the description of the *Golgi apparatus* of the zymogen cell (cf. p. 28 ff.) is applicable to that of the mucoid cell as well. The magnitude of the Golgi apparatus seems to vary greatly from one mucoid cell to another, but in most cases it is roughly of the same size as in the zymogen cells. Also, the thickness of the separate membranes and the interspace between the membranes of a pair are of the same magnitude as in the Golgi apparatus of the zymogen cell. The Golgi vacuoles are as a rule flat and usually do not become as large as in the zymogen cells (Figs. 32–34). Compared with this type of cell the mucoid cells have a greater number of Golgi vesicles. Just as in the case of the zymogen cells, a small number of secretory granules are present in the neighborhood of the Golgi apparatus (Figs. 13, 23, 32). These granules are generally smaller in diameter than other mucoid-cell secretory granules. It may be added that the Golgi apparatus in mucoid cells in most cases tends to be difficult to study in methacrylate embedded specimens because then its membranous structures are rather indistinctly visualized.

Round or polygonal, maximally dense *particles* with a diameter of about 140 Å are very abundant in all parts of the mucoid cells (Figs. 24, 25, 27, 31, 32). Sometimes they are associated with a membrane pair, thereby creating *α-cytomembranes* (Fig. 24). Such membranes, however, are not prominent cytoplasmic features since most of

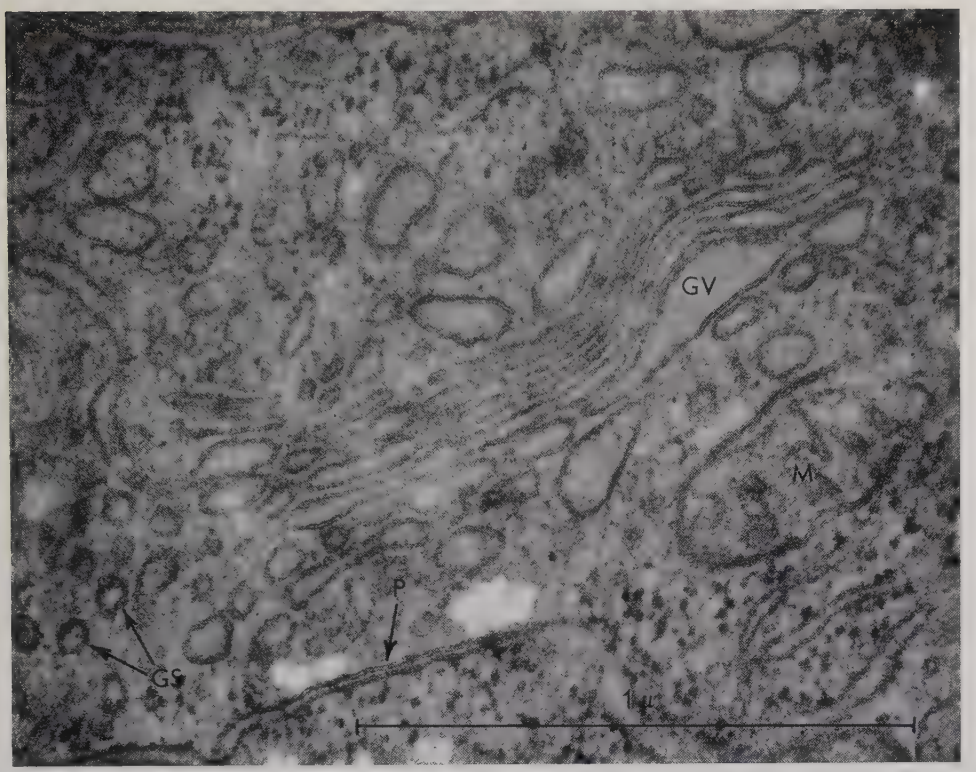


FIG. 33. A mucoid-cell Golgi apparatus. Most of the Golgi vacuoles (*GV*) are rather flat. Peripherally to these, numerous Golgi vesicles (*GS*) are aggregated. Notably, many membranes have a three-layered appearance. *M*, mitochondrion; *P*, plasma membrane. Vestopal embedded section fixed at pH 8.5. ×74,000.

the particles are free and not attached to any membranes. They are then usually found in clusters of four to eight.

*Vacuole-containing bodies* (Figs. 19, 23, 25), *dense microbodies*, and *lipid droplets* are other organelles which occur rarely in the mucoid cells.

The *ground cytoplasm*, the density of which is moderately high, not seldom contains aggregations of closely packed *fibrillar elements* (Figs. 30, 34). These, which apparently occur in all parts of the cytoplasm, occasionally seem to arise from the lateral cell surfaces (Fig. 34). They take an undulating course and are therefore hard to estimate for length. Their thickness does not exceed 50 Å.

As a rule the *nucleus* has essentially the same appearance as the nucleus in zymogen cells (Figs. 13, 23).

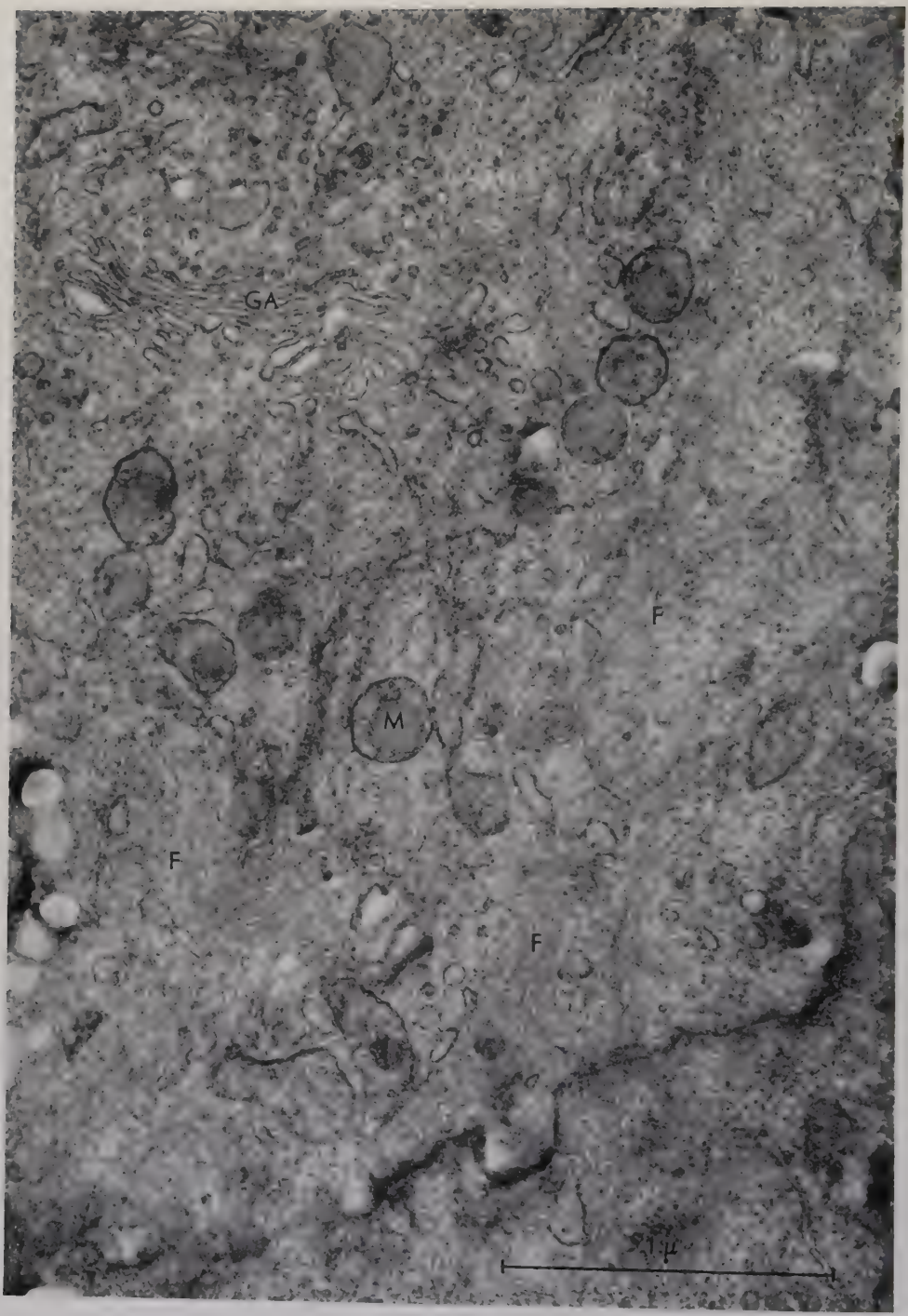


FIG. 34. Mucoid cells with aggregations of closely packed fibrils (*F*), apparently arising from the lateral cell surface in the lower part of the figure. The fibrils are undulating and seem to extend several $\mu$ into the cytoplasm. Mitochondria (*M*) with granules and a Golgi apparatus (GA) composed of membranes, flat vacuoles, and numerous vesicles are also seen in the cytoplasm. Vestopal embedded section fixed at pH 8.5. ×46,000.

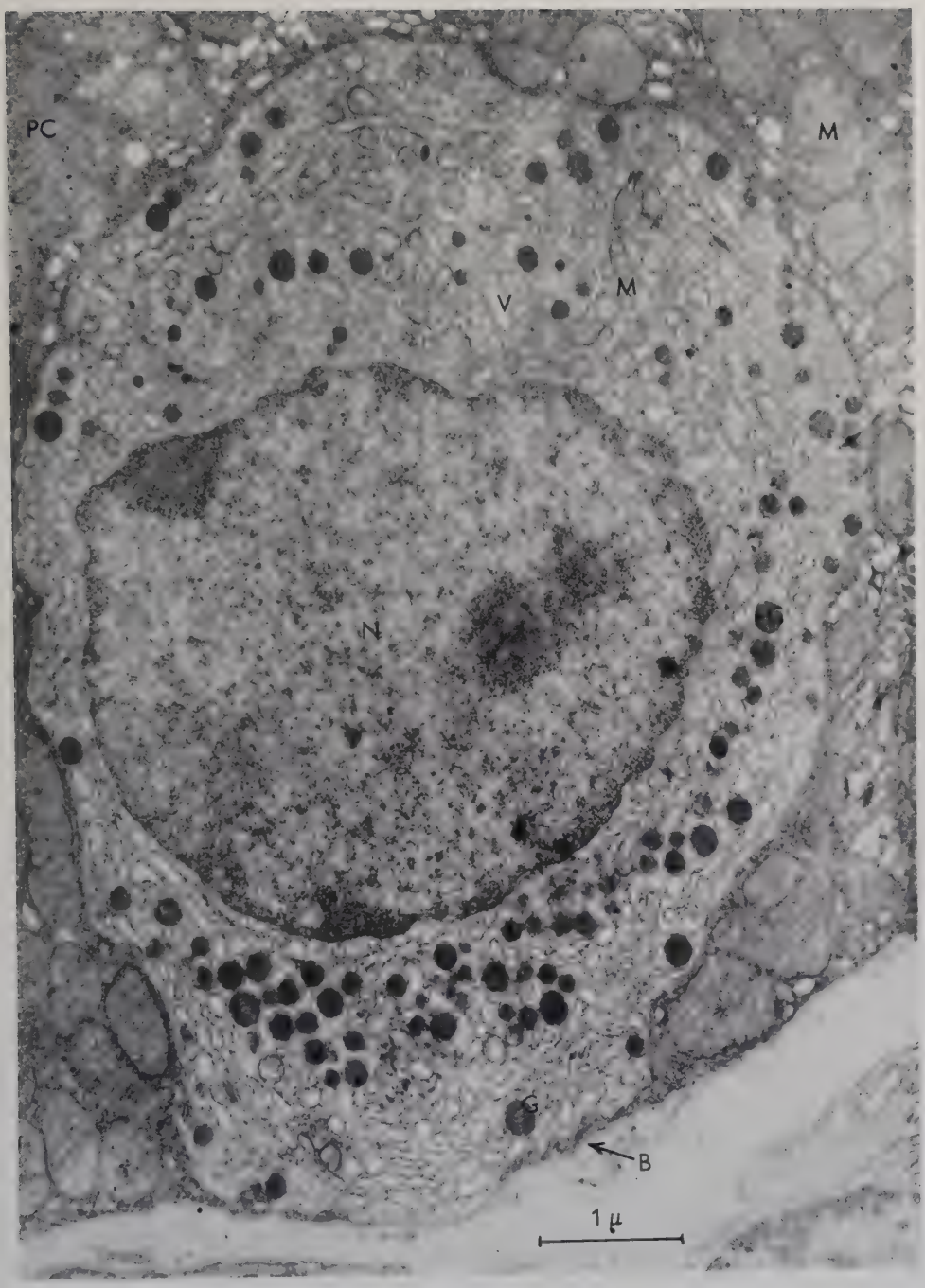


FIG. 35. Survey of an argyrophil cell in the basal parts of which lie most of the secretory granules (*G*). The cytoplasm also contains mitochondria (*M*), vacuoles (*V*), and α-cytomembranes. The density of the ground cytoplasm is considerably lower than in the neighboring parietal cells (*PC*). The cells rest on the basement membrane (*B*). *N*, nucleus. Vestopal embedded section fixed at pH 8.5. ×20,000.

*Argyrophil cells*

Only comparatively few argyrophil cells are encountered in the fundus gland of the mouse. Bordering against the basement membrane basally, their remaining surfaces adjoin other epithelial cells of the gland, mainly zymogen cells, suggesting that the argyrophil cells are more common in the lower than in the upper half of the fundus gland. No argyrophil cell was seen to contact the glandular lumen.

The *plasma membrane* on those surfaces which face towards other epithelial cells is about 60 Å thick and on the whole straight (Fig. 35). It is separated from the latter cells by an intercellular space about 90 Å wide. The plasma membrane on the basal surface of the argyrophil cell is slightly undulating (Figs. 35, 36).

Characteristically, a large number of granules—henceforth called *secretory granules*—are distributed throughout the cytoplasm of the argyrophil cell (Figs. 35, 36). However, most of the secretory granules are found in the cytoplasm between the nucleus and the basal cell surface. As a rule they are round and have a mean diameter of 0.18 $\mu$. A typical secretory granule consists of a central, round, homogeneous core of high density, which is separated from the approximately 40 Å thick dense surface membrane by an annular zone of low density about 80 Å wide (Figs. 36–38).

A varying number of irregularly outlined *vacuoles* are observed scattered all over the cytoplasm. Their largest dimension seldom exceeds 0.5 $\mu$, and their surface membrane is approximately 60 Å thick. The content of these vacuoles has a low density (Figs. 35–37).

Also spread throughout the cytoplasm are a number of oval or elongated *mitochondria* (Figs. 35–38), having a minor axis averaging 0.14 $\mu$ and a maximum measured length of about 1.5 $\mu$. They exhibit a three-layered outer membrane with a dense layer about 30 Å thick on either side of a less dense layer some 60 Å thick. The few inner membranes, as a rule transversally disposed, have a similar composition, the two external dense layers being about 40 Å thick and the less dense central one approximately 70 Å thick (Figs. 36–38). The mitochondrial matrix is of low density.

Basically the ultrastructure of the *Golgi apparatus* in the argyrophil cells is the same as in the zymogen cells (Fig. 37). Its extent is small, however, and of the same order as in the parietal cells. Around the Golgi vacuoles, which are of the same density as the embedding medium, are grouped numerous Golgi vesicles and occasional secretory granules. The latter have a smaller diameter and a wider annular zone than secretory granules elsewhere in the cytoplasm.

In various places of the cytoplasm one sometimes encounters one or more, unstruc-

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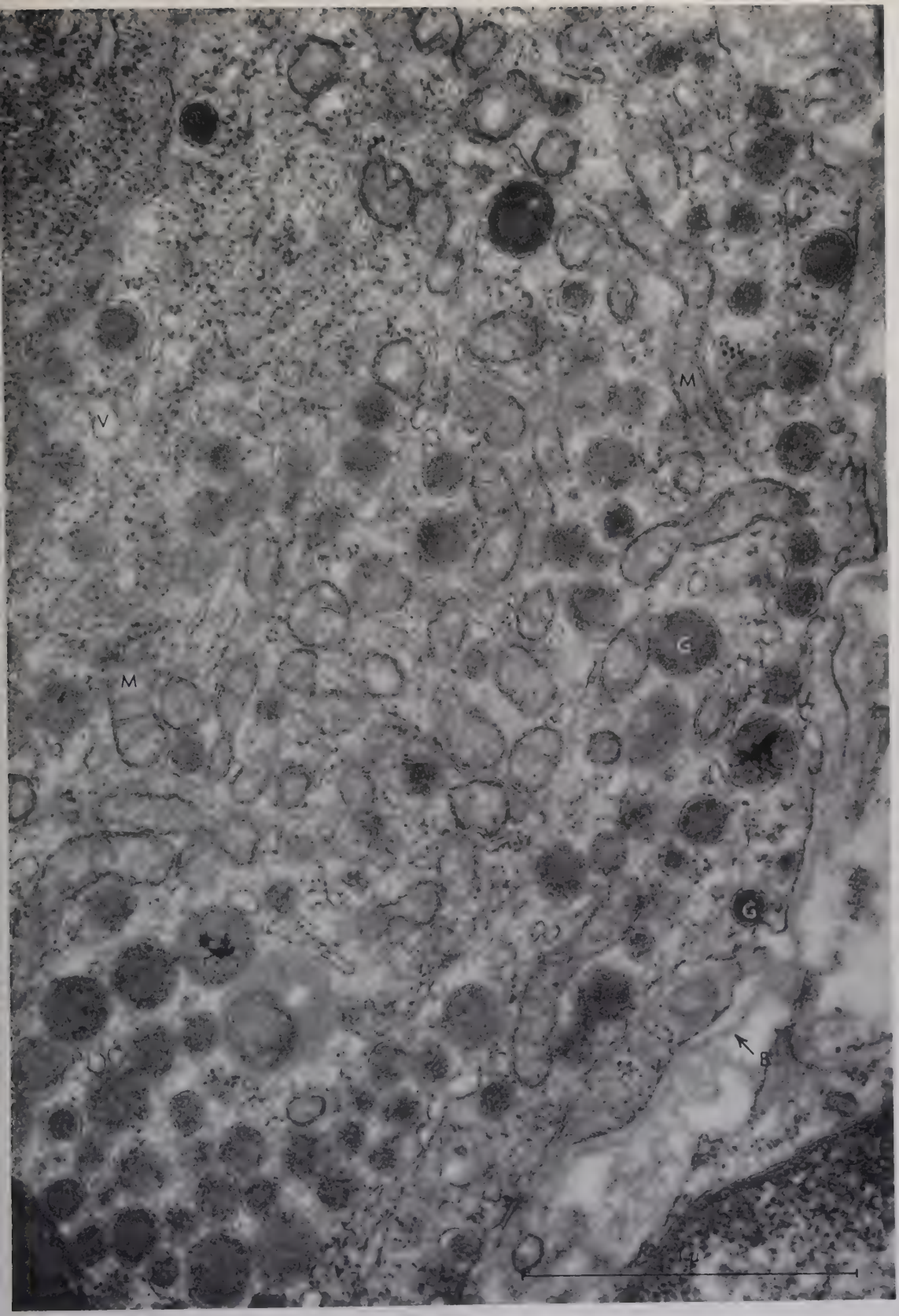
FIG. 36. Part of an argyrophil cell with numerous secretory granules (*G*), mitochondria (*M*), and small particles, as well as a few vacuoles (*V*). *B*, basement membrane. Methacrylate embedded section fixed at pH 8.5. × 56,000.


M
V
M
G
G
B
1 μ

tured areas of grossly round or oval shape and irregular outline (Fig. 38). Henceforth these formations which contain a substance of high density will be known as *pigment bodies*. Their mean diameter ranges from approximately 0.5–1.5 $\mu$. Pigment bodies are surrounded by up to 10 pairs of membranes. Minor, unstructured, less dense zones are sometimes encountered between the inner substance and the nearest membrane. The two membranes of a pair have a thickness of the order of 30 Å and are separated by a less dense zone about 60 Å wide. The distance from one membrane pair to the next usually ranges within the limits 60 to 200 Å.

Furthermore, the cytoplasm is the site of a few small, round or polygonal, maximally dense *particles*. They are often found in groups of four to eight and have an average diameter of 130 Å. Occasionally such particles may be observed to contact a short membrane.

The *nucleus* is round or oval and its outline on the whole regular. Otherwise its ultrastructure agrees well with that of the zymogen cell nucleus (Figs. 35, 37).

## Vestopal Embedded Tissue

Almost all the Vestopal embedded tissue examined in the present investigation was fixed at pH 8.5; merely a few blocks of Vestopal embedded tissue fixed at pH 7.2 were studied for comparative purposes (Figs. 19, 31). It appeared that the epithelial cells of the fundus glands exhibited a highly varying ultrastructural picture after Vestopal embedding and fixation at pH 7.2. In the parietal cells, which were particularly susceptible to such variation, the distance between the cytoplasmic organelles in some cases was remarkably large, in other cases the corresponding distances were smaller, of the same order as in methacrylate embedded, well-preserved tissue. Sections of Vestopal embedded tissue fixed at pH 8.5 very seldom displayed any similar variations. Among the various intracellular organelles, only the zymogen granules showed significant ultrastructural dissimilarities after fixation at these two pH levels. Thus, in most cases the zymogen granules had a greater density after fixation at pH 7.2 than after fixation at pH 8.5. The observations described in the following were derived from Vestopal embedded tissue fixed at pH 8.5.

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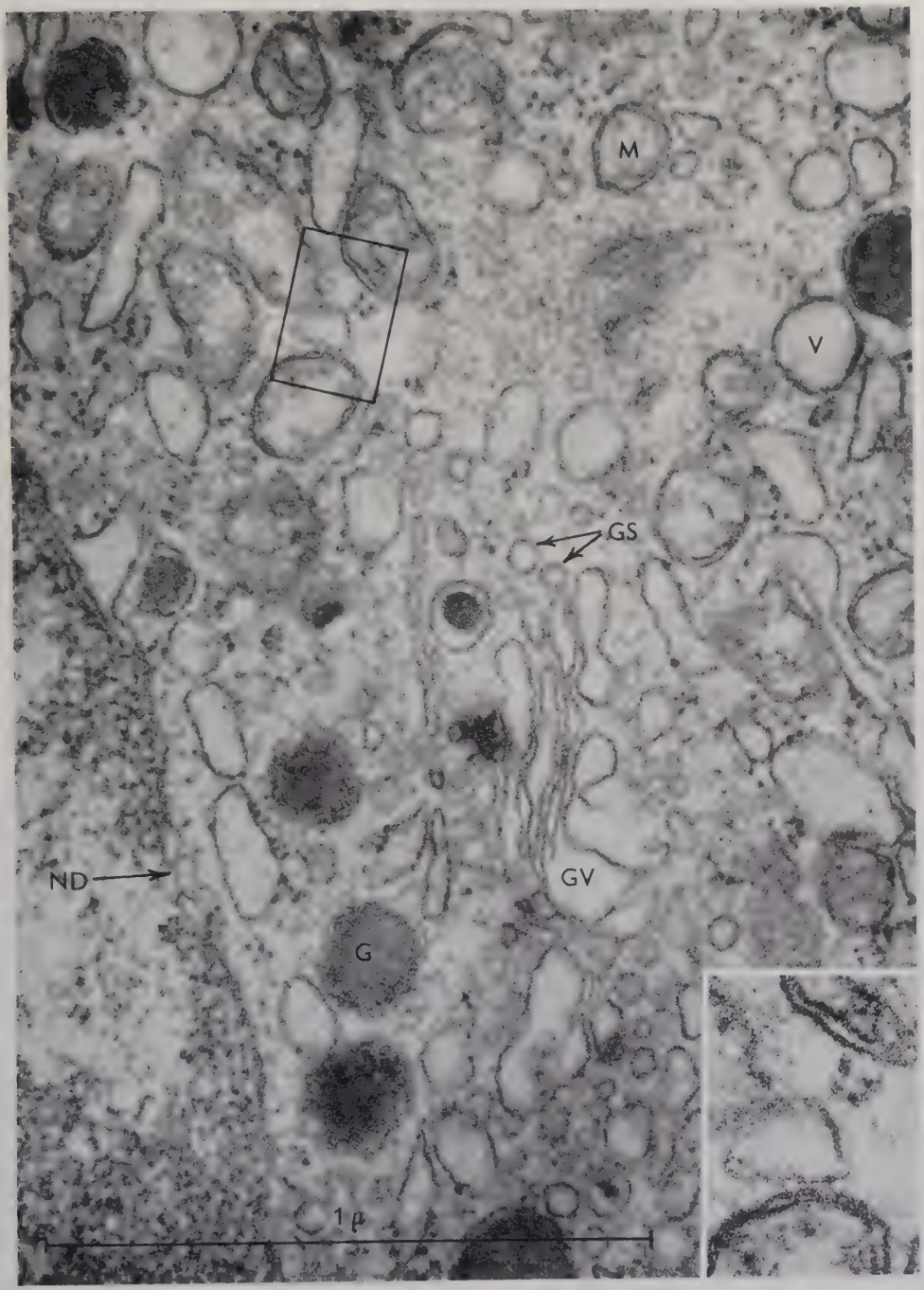
Fig. 37. Part of an argyrophil cell. To the left lies part of the nucleus whose three-layered surface membrane exhibits a discontinuity (*ND*), apparently bridged by a thin membrane. The Golgi apparatus in the centre comprises membranes, a few vacuoles (*GV*), and, farther peripherally, some vesicles (*GS*). The secretory granules (*G*) have a round core of high density separated from the surface membrane by an annular zone of low density. Compared with those elsewhere in the cytoplasm, the secretory granules near the Golgi apparatus are smaller and have a broader annular zone. A few mitochondria (*M*) and vacuoles (*V*) are also present. The inset shows the area in the rectangle in greater detail. The two partly visible mitochondria have outer membranes whose external dark layer appears to be made up of three lamellae. Vestopal embedded section fixed at pH 8.5. × 84,000; inset, × 170,000.


M
V
GS
ND
GV
G
1 μ

Ultrastructurally Vestopal embedded tissue deviates in some fundamentally important respects from similar tissue embedded in methacrylate and fixed at the same pH. Among other things, this is manifested by differences between the dimensions of the various organelles, as appears from the data compiled in Tables I and II.

The appearance in the electron microscope of Vestopal embedded tissue differs most strikingly from that of methacrylate embedded tissue in having a considerably narrower density spectrum. As a rule the ground cytoplasm and the matrix of the various organelles differ only slightly in density from the background. However, one sometimes observes irregularly scattered and sharply demarcated areas of empty appearance. These areas are usually found within membrane-bound regions, as for example some of the vacuoles in parietal cells and occasional regions between closely spaced microvilli (Figs. 14, 43, 44).

Another feature typical of Vestopal embedded tissue is the distinct delineation of all membranous structures, one manifestation of this being the much higher proportion of membranes revealed as multi-layered than in methacrylate-embedded tissue (Figs. 8, 22, 33, 37, 41, 44).

Only those formations which differed in structure and/or dimensions from corresponding formations in methacrylate embedded specimens fixed at pH 8.5 will be considered.

*Zymogen cells*

Not only on the apical but also on the lateral and basal surfaces, the *plasma membrane* is often seen to be composed of three layers (Fig. 41).

The *zymogen granules* contain a finely granular and moderately dense substance of fairly constant appearance. Occasionally, however, zymogen granules containing a denser core are observed (Fig. 40). The approximately 60 Å thick and often discontinuous surface membrane is not seldom visualized as composed of three distinct layers, with two dense layers on either side of a less dense layer, all of which are of roughly equal thickness (Fig. 8). The mean diameter of the zymogen granules in Vestopal embedded tissue is about 1.04 $\mu$, a value significantly greater than the corresponding figure of 0.65 $\mu$ for methacrylate embedded tissue.

The *Golgi vacuoles* exhibit a finely granular and moderately dense appearance (Fig. 3).

*Parietal cells*

The *microvilli* on the apical and canalicular cell surfaces seem coarser than in methacrylate embedded tissue (Fig. 14). Their average diameter of some 960 Å is significantly greater than in methacrylate where it averaged 810 Å.

The aforementioned cell surfaces are equipped with a three-layered *plasma membrane* (Fig. 44). The thickness of the dense layers is of the order of 20 Å and that of

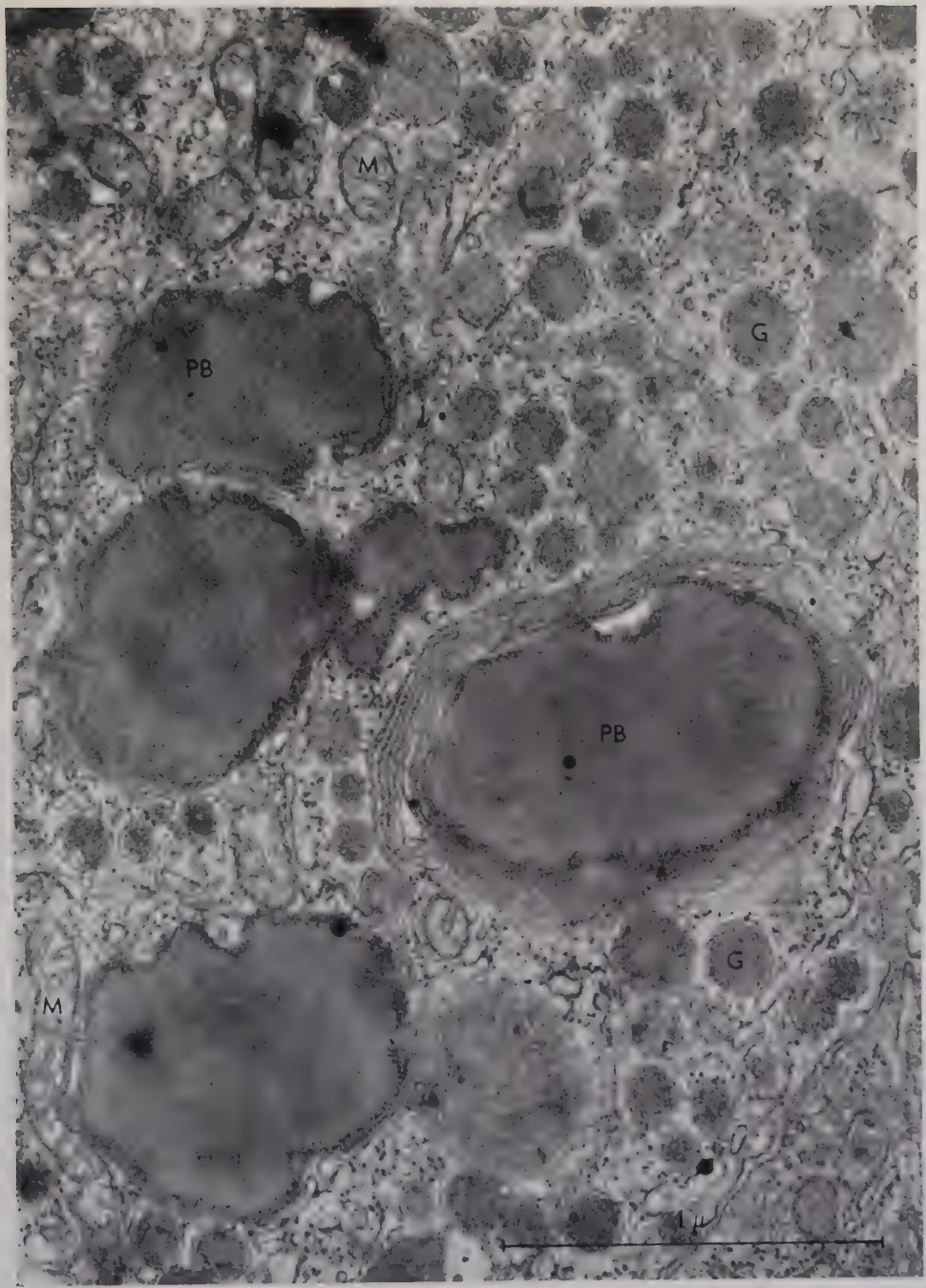



FIG. 38. Part of an argyrophil cell. The cytoplasm contains several pigment bodies (*PB*) surrounded by a varying number of concentric membrane pairs. Moreover, secretory granules (*G*) and mitochondria (*M*) may be observed. Vestopal embedded section fixed at pH 8.5. × 52, 000.

the less dense layer about 40 Å. On the lateral and basal surfaces, too, the plasma membrane is often depicted as a three-layered structure.

More distinctly than in methacrylate embedded tissue, the surface membrane of the *vacuoles* appears as a multi-layered structure, comprising a less dense layer some 40 Å thick between denser layers about 20 Å thick (Figs. 22, 44). Some of the vacuoles have an undulating outline. The diameter of the vacuoles averages 0.13 $\mu$ a figure not significantly different from the value of 0.14 $\mu$ in methacrylate.

The organization and dimension of the *mitochondria* agree well with those in methacrylate sections. Sometimes, however, one observes a subdivision of the dense layers of the mitochondrial inner membranes into two dense lamellae separated by a less dense lamella, each one having a thickness of the order of 15 Å.

The *Golgi apparatus* is often more distinctly visualized in Vestopal sections than in methacrylate sections (Figs. 14, 21).

Those portions of the *cytoplasm* which are the most dense in methacrylate sections (marginally along the apical and basal cell surfaces and near the intracellular canaliculi) contain a much higher proportion of finely granular, moderately dense substance than the cytoplasm elsewhere in Vestopal embedded tissue fixed at pH 8.5 (Fig. 14).

*Mucoid cells*

Owing to the distinct visualization of their membranes, the mucoid cells exhibit a more easily interpreted ultrastructure in Vestopal than in methacrylate.

Not only on the apical surface, but also on the lateral and basal surfaces does the *plasma membrane* appear as a three-layered structure (Fig. 33).

In Vestopal embedded sections the majority of *secretory granules* contain a homogeneous substance of low density, and they seem to be less closely spaced than in methacrylate. They have a mean diameter of some 0.27 $\mu$, in this respect not differing significantly from the methacrylate embedded ones. In most secretory granules one observes a narrow, moderately dense belt separated from the surface membrane by a thin, less dense interspace (Fig. 29).

With an average length of some 0.21 $\mu$, the minor axis of the *mitochondria* is not significantly different from that of the methacrylate embedded mitochondria, namely 0.16 $\mu$. The inner membranes appear much more distinctly than in methacrylate (Figs. 24, 29).

The small maximally dense *particles* associated with membrane pairs of varying lengths are encountered more often than in methacrylate sections.

*Argyrophil cells*

Apart from the more general deviations mentioned in the introduction to this chapter, Vestopal and methacrylate embedded argyrophil cells display no significant differences (Figs. 35, 37).

## CHAPTER 4

# Discussion

## COMMENTS ON MATERIALS AND METHODS

The writer's findings deviate in some respects from those of previous workers. Such deviations may be due to differences between species but could also arise from variations in the composition of the food or in other external factors.

One important factor which by comparison with other investigations may have differed is the functional state of the gastric mucosa. Before being sacrificed, the experimental animals used by most workers had been starved for varying lengths of time but in most cases it is not specified how strict the fasting was. When rats or mice have been used as experimental animals, the measures taken to preclude coprophagia seem to have been inadequate. In some investigations, moreover, the animals had been anesthetized before excision of the tissue, and then the possibility cannot be ruled out that anesthesia had influenced the functional state of the gastric mucosa.

Another important factor in this connection must certainly be the manner in which the excised tissue is fixed and embedded. These procedures have varied greatly from one investigation to another.

All previous workers have used fixation fluids with pH-values in a narrow range (cf. p. 86) but incorporating different buffers, in most cases veronal acetate (*73*, *78*, *84–86*, *99*, *109*, *115*, *128*, *191*, *204*), and occasionally potassium dichromate (*24*, *115*), potassium phosphate (*182*, *183*, *186*, *187*, *191*) or s-collidine (*99*, *120*, *121*). Furthermore, the tonicity of the fixation fluid has apparently varied very much indeed. Thus, in some investigations the fixation fluid has been isotonic relative to blood (*73*, *76*, *78*, *84–86*), in others hypotonic (*121*, *128*, *187*, *204*) or hypertonic (*99*, *115*). There has also been some variation in the dehydration procedure. Whilst methacrylate has generally been used for embedding the tissues, occasional workers have instead used Araldite (*115*) or Epon (*120*, *121*).

The writer has not attempted to evaluate the extent to which the aforementioned factors could be responsible for differences between published results. However, as is clearly evident from previous investigations on other tissues (*168*, *213*), conspicuous

effects on the electron microscopic appearance of a tissue are induced by deviations of fixation fluid osmolality from blood isotonicity.

In the present investigation the quality of the preservation of the cells in a number of blocks had to be rated as poor. Among the features interpreted as signs of poor preservation are the occurrence of vacuolized, occasionally swollen mitochondria with broken inner membranes, and clumping of the ground substance of the nucleus and cytoplasm, whereby a coarsely granulated appearance was imparted to them (cf. *163*, *168*, *213*). The writer's assessment on the basis of these criteria of the qualitative effectiveness of the fixation had the result that in the series where the pH of the fixation fluid was 7.2 and the tissue was embedded in methacrylate more than 80% of the experimental mice had to be discarded. This poor yield was probably due to the fact that the presence in the stomach of pepsin and hydrochloric acid simultaneously is highly conducive to the development of autolytic lesions. Judging by published electron micrographs, it is reasonable to suspect that a succession of previous workers have based their descriptions of the ultrastructural organization of epithelial cells in the fundus glands on observations made in poorly preserved cells.

The methods adopted for the present investigation were selected in the light of results of earlier authors' trials designed to evaluate how the quality of the preservation of various cells was affected by a variety of factors, especially: (1) undelayed fixation, and (2) blood-isotonicity of the fixation fluid. Moreover, the writer's observations have manifested the advantages accruing from the use of (3) an alkaline fixation fluid of high buffering capacity, and (4) an embedding medium other than methacrylate.

## DISCUSSION OF RESULTS

In the following will be discussed the ultrastructure of some of the organelles in the various epithelial cell types in the fundus glands. Unless otherwise stated, this discussion is based on findings applying to methacrylate embedded tissue, after fixation at pH 7.2 for zymogen cells and parietal cells and at pH 8.5 for mucoid cells and argyrophil cells. Some of the morphological findings will also be considered from physiological and biochemical aspects. Next, those ultrastructural manifestations will be analyzed which appear when embedding medium and/or pH and buffering capacity of fixation fluid are altered. Finally, a brief outline will be given of the principles and criteria employed in establishing the identity of the various types of cell.

## Methacrylate Embedded Tissue

*Cell surfaces*

The structure here called the *plasma membrane* is represented on electron micrographs by a line extending around the entire surface of a cell. At high resolutions it is visualized as a structure composed of two dense layers and an intermediate less dense layer. This writer's findings neither confirm nor refute previous hypotheses regarding the definition and composition of the plasma membrane (e.g. *173*, *195*).

The triple layering of the plasma membranes of the exocrine cells in the fundus gland appears most distinctly on the apical surfaces. Each of these layers, the dimensions of which are roughly the same in all the cells, has a thickness of the order of 25 Å. A similar three-layered composition has been reported for the plasma membrane of other glandular epithelial cells (e.g. *168*, *170*, *178*, *213*), the thickness of the component layers varying between 20 and 40 Å.

On the lateral surfaces of the exocrine cells of the fundus gland the plasma membrane appears to be somewhat thinner than on the apical cell surface. The thickness ratio between the apical and lateral plasma membranes varies in previous observations in other glandular epithelial cells preserved by the same, or nearly the same, method as that in the present investigation. Thus in the columnar absorbing cells of the jejunum the lateral plasma membrane was only about two-thirds as thick as the apical plasma membrane (*213*); in the epithelial cells of the thyroid gland the opposite was the case, the plasma membrane on the apical surface being thinner (*53*); and in the cells of the proximal renal tubules and of the tracheal epithelium, lastly, the lateral and apical plasma membranes were approximately equally thick (*168*, *170*).

Here it is necessary to stress that measurements of structural dimensions of this magnitude may be highly inaccurate (*172*, *211*), and the errors of measurements obtained by different measuring methods are unlikely to be consistent. Hence, the dimensions of the plasma membranes reported by previous workers are not always directly comparable.

Yet the aforementioned variations in plasma membrane thickness may quite conceivably be real enough. Such variations could be morphological manifestations of dissimilar chemical composition (cf. *174*). For example, as demonstrated by Finean (*66*, *67*), the thickness of the layers in the myelin sheath is influenced by their degree of hydration prior to fixation. Perhaps a similar mechanism could cause differences in plasma membrane thickness between one cell surface and another.

The exocrine cells of the fundus glands have *terminal bars* with the same ultrastructural organization as in other glandular epithelia (e.g. *53*, *62*, *168*, *213*). In the intercellular space apical to the terminal bar region one occasionally observes a thin, dense stratum, which apparently represents the fused outermost plasma

membrane layers of two adjacent cells. In the fundus gland this stratum is visualized most distinctly between two adjacent zymogen cells. Similar strata are also encountered between closely packed microvilli of parietal cells (cf. Fig. 44), and have been observed previously in other tissues (*102*, *103*, *134*). These strata might be of the same nature as the so-called intraperiod line of myelin sheaths (*173*). Wherever hitherto observed these strata have similar dimensions.

Often the lateral cell surfaces of adjacent zymogen cells or adjacent mucoid cells are the site of formations closely resembling terminal bars but lying farther away from the apical cell surfaces. These formations most likely correspond to the so-called *desmosomes* or, as they are also called in the light microscopic literature, intercellular bridges. Under the light microscope they have been observed in the fundus glands both between zymogen cells (*106*) and between surface epithelial cells (*147*). In the fundus glands only the mucoid cells contain appreciable amounts of fibrillar elements resembling those seen radiating into the cytoplasm from both terminal bars and desmosomes in other cell types (*62*, *146*).

It has been stated that whereas terminal bars are continuous structures encircling the entire cell near its apical surface, desmosomes constitute discrete regions scattered over the lateral cell surfaces (*62*). Considering that, in the present investigation, terminal bars were invariably present in sections through the region where the apical and lateral epithelial cell surfaces meet, it is highly probable that the fundus-gland terminal bars are also continuous structures existing around the whole perimeter of the individual cells. The observations made in the present study do not refute the view that the desmosomes are strictly localized structures.

Although they vary in number and appearance with the type of cell, *microvilli* are observed on the apical surface of all the exocrine cells of the fundus gland. Morphologically the microvilli of these cells do not differ conspicuously from the microvilli of other cells (e.g. *53*, *168*, *178*, *213*). Notably, however, the apical surface of the mucoid cells often had only a small number of short, stubby cytoplasmic processes.

In parietal cells microvilli also occur on the walls of the intracellular canaliculi. Like other parts of the apical and canalicular cell surface, they are bounded by a plasma membrane which, at high resolution, is visualized as a three-layered structure. Hally (*74*, *78*) described an extra membrane lying beneath the ordinary plasma membrane in the microvilli of parietal cells. No such extra membrane was encountered in the present investigation. In thick sections, however, cytoplasmic granular or filamentary elements inside the microvilli could simulate a membranous structure. But the individual filamentary and granular elements can readily be distinguished in thin sections at high resolution.

Though only some of the *intracellular canaliculi* of the parietal cells can be traced

to their opening on the apical cell surface, it is probable that all the variously sectioned canalicular portions seen in the cytoplasm eventually open at the apical cell surface. By virtue of these canaliculi and the numerous microvilli on the apical as well as on the canalicular cell surfaces, the parietal cell seems to have a larger surface area in functional contact with the glandular lumen than any other secretory cell ever examined. Hally (*74*) estimated the area of this surface to some 500 $\mu^2$ per cell: until reconstructions of serial sections have been made, however, any estimate of the magnitude of this area will be very uncertain.

Many authors have drawn attention to the multiple significance of the intracellular canaliculi, most having emphasized their role as a drainage system for the secretory products of the parietal cells (*31*, *74*, *91*, *111*). It is generally assumed that these cells secrete hydrochloride acid into the intracellular canaliculi, and, it has been suggested that the hydrochloride acid is formed at or very near the walls of these canaliculi (*29*, *41*, *91*). Although the importance of a large secretory surface has been pointed out (*74*), some further discussion seems justified.

On the basis of results from combined electron microscopic and histochemical investigations a number of workers have claimed that a variety of enzymes are localized to the plasma membrane or its immediate vicinity, the most marked histochemical reactions being obtained along that part of the cell surface across which the main secretory activity is supposed to take place. Positive reactions have here been reported for adenosinetriphosphatase (ATP-ase) (*58*, *164*, *199*), 5-nucleotidase (*58*), acid phosphatase (*190*), and alkaline phosphatase (*18*, *28*, *136*). Although the reliability of their methods can be questioned (*7*, *28*), these authors' observations open up new and theoretically interesting aspects. Accordingly, provided that the aforementioned enzymes are structurally associated with these plasma membrane regions and that their concentrations are approximately proportional to the surface area of these regions, the plasma membrane on the apical and canalicular surfaces of the parietal cell could accommodate very large amounts of enzymes.

Biochemical investigations have shown that the immense energy requirements of hydrochloride acid production are provided directly or indirectly from adenosinetriphosphate (ATP) and other high-energy phosphates (*41*). The large cellular surface in functional contact with the glandular lumen could thus be a means for the parietal cells to lodge the large quantity of enzymes which are needed to metabolize these high energy compounds at the required rate.

*Intercellular lacunae*

In the fundus glands of the mouse, the width of the intercellular space exhibits major variations only along the mucoid cells. Shibasaki (*191*) observed similar irregularities in the rat and called them intercellular canaliculi. He could not definitely

establish whether or not these intercellular canaliculi opened into the lumen of the gland. Intercellular spaces or lacunae of the same type have been perceived in a number of other tissues (e.g. *170*, *178*, *213*).

Various theories have been advanced concerning the functional significance of such lacunae. Shibasaki (*191*) assumed that they were correlated to the absorptive function of the gastric surface epithelium. Rutberg (*178*), however, ascribed the wide intercellular spaces adjoining the acinar parotid cells to morphological adaptation, the cellular surface area being increased to enhance the absorption of substances from the interstitial medium.

Other possibilities suggest themselves. Those mucoid cells which adjoin the intercellular lacunae always have a very dense ground cytoplasm, which makes separate organelles hard to distinguish. The nuclei of these cells resemble what light microscopists call pyknotic nuclei. The author has interpreted this appearance as a sign of impaired cellular vitality, an attitude borne out by the presence of markedly transformed organelles in the cytoplasm of these cells and in the lacunae between them. In the author's opinion, therefore, it cannot be ruled out that the existence between some of the mucoid cells of intercellular lacunae is but one aspect of a process which leads to the necrosis and desquamation into the gastric cavity of these cells. Signs of large-scale desquamation of mucoid cells have been encountered in the fundus glands in light microscopic and autoradiographic investigations (*96*, *130*, *131*, *200*).

*Secretory granules*

The assumption that granules found in zymogen cells and mucoid cells constitute secretory granules, in other words contain products to be excreted, seems justified by light microscopic evidence (*9*, *83*, *112*). Also, in the light of electron microscopic observations on granules in cells of similar appearance (*47*, *59*, *60*) it seems highly probable that the granules of the argyrophil cells constitute secretory granules.

The electron microscopic appearance of the secretory granules varies a good deal from one type of epithelial fundus-gland cell to another. In the zymogen cell the diameter of the secretory granules averages three-fourths of a micron, in the mucoid cell it is about one-third of a micron, and in the argyrophil cell it scarcely attains a fifth of a micron. The parietal cell, lastly, completely lacks secretory granules. Moreover, the distribution of the secretory granules varies between cell types. Thus, in argyrophil cells they are scattered throughout the cytoplasm, in zymogen cells they are found in the supranuclear and paranuclear portions of the cytoplasm, and in mucoid cells they are mainly confined to a marginal zone along the apical cell surface.

Ever since Heidenhain (*83*), Langley (*112*), and Langley and Sewall (*113*) published their pioneering findings, the consensus of opinion has been that the secretory granules in the *zymogen cells* contain pepsinogen, a pre-stage of pepsin. The likelihood of this assumption has been confirmed time and again (e.g. *14*). Furthermore, it has earlier been proposed that the rennin content of the gastric juice is derived from the zymogen granules (*112*). These granules could also be the source of the lipase secreted from the gastric mucosa. If this is correct the granules of the zymogen cells are very similar to those of the pancreatic acinar cells which have been demonstrated to contain several different enzymes (cf. *158*).

Most probably the secretory granules of *the mucoid cells* contain mucin-like substances (cf. *8*, *83*).

The secretory products of the *argyrophil cells* of the gastric mucosa have been suggested to contain, among other things, glucagon (*201*) or serotonin (*57*). Histological staining reactions favor the view that the content of the secretory granules of the argyrophil cells is of catechol nature. However, its exact composition is still being debated (cf. *122*).

*Vacuoles*

Among the epithelial cells of the fundus glands, only the *parietal cells* contain vacuoles in considerable numbers. These vacuoles have a diameter of approximately 0.14 $\mu$.

When Ito (*98*, *99*) studied the ultrastructure of the parietal cells in a large number of species, he encountered merely a few vacuoles. Instead he observed a great number of tubular elements measuring from 200 to 500 Å in diameter. These formations, which were often interconnected, were considered to belong to the agranular endoplasmic reticulum. He expressed the view that the vacuoles in the parietal cells were artefacts, probably produced by swelling and vesiculation of this agranular endoplasmic reticulum. Similarly, Lawn (*115*) occasionally observed vacuoles interconnected with small tubules. Conversely, neither Kurosumi *et al.* (*109*) nor Hally (*74*, *78*) were able to find any evidence of interconnections between the vacuoles.

That the vacuoles observed by the writer in the cytoplasm of the parietal cells might be artefacts due to vesiculation of an agranular endoplasmic reticulum of tubular shape is a possibility which cannot be entirely ruled out. But this eventuality would seem to be at least partly contradicted by the fact that precisely the same type of ultrastructural appearances was obtained regardless of how the tissue was fixed and embedded. The fixation methods used in the present investigation were of the chemical type, however, and artefacts could arise in the tissue before and during its exposure to the fixatives.

Although Ito (*99*) considers it less likely, it is possible that the vacuoles are a true representation of the structures in the living parietal cell, and that preparatory maltreatment of the cells transforms the vacuoles into tubules. It appears that this might have happened in the tissue specimens which Ito examined. Thus, the use of a hypertonic fixation fluid[1] could easily give rise to shrinkage of the vacuoles so that in the sections they would convey the impression of being tubular structures. Zetterqvist (*213*) thus found that the vacuoles in the jejunal columnar absorbing cells were clearly visualized after fixation in a blood-isotonic agent but shrank so as to become barely discernible after fixation in a hypertonic medium.

Many workers have regarded the vacuoles of the parietal cells as a part of the smooth-surfaced endoplasmic reticulum (*73*, *76*, *98*, *99*, *109*, *115*, *148*, *155*, *183–185*, *191*). However, this classification is not generally accepted (*74*, *187*). Considering that the smooth-surfaced endoplasmic reticulum is a concept defined as a continuous network of membrane-bound cavities (*155*), the vacuoles of the parietal cells should not be included therein until it has been established beyond doubt that they communicate with one another. For the time being these vacuoles should rather be looked upon as a specific type of organelle with the same status as other intracellular organelles.

Like the parietal cells, a series of other cells has numerous vacuoles in its cytoplasm. Among cells of this category mention may be made of the dark cells in the arched collecting tubules of the kidney (*168*, *169*), the ependymal cells in the chorioid plexus (*126*, *133*), the inner fascicular cells of the adrenal cortex (*211*), and the non-pigmented cells of the ciliary epithelium from acetazol-treated animals (*92*). Despite their rather dissimilar functional duties, these cells seem to have in common that they elaborate protein-poor materials.

In the *mucoid cells* the vacuoles are located close to the lateral cell surfaces. They are smaller and markedly less numerous than the vacuoles in the parietal cells. Being disposed in single rows, apparently arising from invaginations in the lateral plasma membrane, they give the impression of being pinched-off from the lateral plasma membrane. Hence they closely resemble the pinocytotic vesicles of the capillary endothelial cells (*151*).

Vacuoles of similar appearance have also been encountered in close relationship to the lateral cell surfaces in the non-pigmented ciliary epithelium (*161*). In analogy with the assumed function of the vacuoles in these cells (*161*), the vacuoles of the mucoid cells could serve as vehicles for transporting material from the intercellular space to the cytoplasm.

[1] Ito's *s*-collidine-based fixation fluid yields a freezing point depression of about 0.70°C, that is an approximately 25 % higher value than those of the two blood-isotonic fixation fluids used in the present investigation.

*Mitochondria*

The dimensions and appearance of the mitochondria vary considerably from one type of cell to another in the fundus gland.

Many mitochondria in *zymogen cells* are visualized in the electron micrographs as very elongated formations. Some of the round and oval mitochondrial contours in the sections probably constitute elongated mitochondria which have been sectioned perpendicularly or obliquely to their long axis. However, the round or oval mitochondria are encountered much more frequently. Assuming a random orientation of the mitochondrial long axis in relation to the sectioning plane the observed proportions of elongated, oval and round mitochondria fit well with the conception that the majority of mitochondria in zymogen cells have an elongated shape (cf. *209*).

Pioneering in the modern electron microscopic study of *parietal cells*, Sedar (*182*) observed the presence in these cells of organelles containing inner membranes and thereby proved that they were true mitochondria. The inner membranes are very closely spaced. Hally (*74*) and Lawn (*115*) reported an average of 30 inner membranes per micron. In the present investigation the inner membranes of the mitochondria in the parietal cells sometimes reached a frequency of 40 per micron, an exceptionally high figure compared with mitochondria in other cells.

A feature characterizing the ultrastructure of many mitochondria in the *mucoid cells* is the presence of one or more large, homogeneous granules which occupy a large proportion of the mitochondrial volume. Such mitochondrial granules have never been seen in any of the other epithelial cells in the fundus gland. But similar large, homogeneous granules have been observed previously in the mitochondria of the uterine epithelium during the secretory phase (*141–144*), in cells of hyperplastic epidermis (*69*, *188*) and in testicular interstitial cells (*26*).

The mitochondria in the *argyrophil cells* seem to lack the opaque 300 Å particles characteristic of the mitochondria in most other cells but exhibit no other peculiarities. They have a smaller diameter than the mitochondria in the other epithelial cells of the fundus gland.

So far as their outer and inner membranes are concerned, the mitochondria in the various epithelial cells of the fundus gland are fundamentally very similar to the mitochondria in cells of most other tissues (cf. *2*, *193*).

Extensive investigations have been concerned with how the mitochondria are involved in cell metabolism (for a review see *117*). It may be assumed, for excellent reasons, that a series of electron transporting enzymes are attached to the mitochondrial inner membranes. The mitochondria further contain enzymes which participate in Krebs' citric acid cycle. However, these enzymes are not firmly bound to the mitochondrial membranes. That the cytochrome *c* content of a tissue may be well correlated to the number of inner membranes in its mitochondria has been postulated

by Palade (*149*). In Dempsey's (*46*) opinion the mitochondria from tissues having high oxidative rates have many inner membranes, whereas the mitochondria from more sluggishly respiring cells have fewer. Another quantitative correlation was discovered by Edwards and Ruska (*50*) when they examined various flight muscles of certain insects. They observed that the mitochondria were more abundant in the more active muscles than in the less active ones.

Against this background, the high number of mitochondria and their great abundance of inner membranes in the parietal cell suggest that this cell has a high enzyme content and a high rate of oxidative metabolism. Such a hypothesis, which has been suggested by earlier authors (*74*, *78*, *115*, *186*, *187*), is strongly borne out by biochemical findings. Thus Bradford *et al.* (*16*) demonstrated that the cozymase and total nicotinic acid concentrations were between 4 and 100 times higher in the parietal cells than in any of the other cell types studied, including other cells of the gastric mucosa.

It has been shown that the hydrochloric acid production of the stimulated gastric mucosa is very well correlated to its oxygen consumption (*39*) but the exact ratio between these rates is a moot point (cf. *38*, *42*). In any circumstances, the parietal cells should be, as emphasized by Crane *et al.* (*32*), among the most active cells in the whole body.

*Golgi apparatus*

On the basis of the results of an electron microscopic study of the acinar cells of the pancreas, Sjöstrand and Hanzon (*197*) were able to define the basic structure of the Golgi apparatus. According to these workers, the Golgi apparatus is composed of *Golgi membranes* demarcating irregularly shaped vacuolar spaces, and of *Golgi granules* whose size and density vary widely. These formations are located in a homogeneous matrix called the *Golgi ground substance*. It is generally accepted nowadays that the Golgi apparatus in all cells has basically the same organization. Any deviations exhibited by different cell types are due to inequalities in the extent of the various components of the Golgi apparatus. Instead of the term Golgi granules a number of authors have used Golgi vesicles. In view of the appearance of the corresponding organelles in the epithelial cells of the fundus gland, the designation *Golgi vesicles* is preferable for the purposes of the present investigation too. Golgi ground substance is not conspicuous in the fundus-gland cells.

In the fundus glands the Golgi apparatus is most extensive in the zymogen cells and the mucoid cells. The Golgi apparatus in zymogen cells is very similar to that in the acinar cells of the pancreas (*197*). Unlike the zymogen cells, the mucoid cells include a large number of Golgi vesicles. In the parietal cells, on the other hand, the Golgi apparatus has so diminutive dimensions that some workers have over-

looked it altogether (*74*, *76*, *109*). The Golgi apparatus in the argyrophil cells is of approximately the same size as in the parietal cells.

In Hally's (*76*) opinion the Golgi apparatus of the parietal cells is composed of the numerous cytoplasmic vacuoles. The objection may be raised that his procedure for estimating the extent of the Golgi apparatus—post-osmication—cannot be considered sufficiently specific. For, as appears from Hally's paper, post-osmication blackens not only the vacuoles but also the mitochondria and the vacuole-containing bodies, the two latter structures definitely falling outside the electron microscopic definition of the Golgi apparatus.

In this connection it should be pointed out that some other cell types in which the Golgi apparatus occupies a comparatively small space have an abundance of vacuoles in their cytoplasm. This combination is thus present in the inner fascicular cells of the adrenal cortex (*211*) and the non-pigmented ciliary epithelium (*92*). In the case of the adrenal cortex cells, too, it has been inconclusively debated whether or not the Golgi apparatus included the numerous cytoplasmic vacuoles (cf. *211*).

*α-Cytomembranes and cytoplasmic particles*

The quantity of α-cytomembranes varies a great deal from one type of epithelial cell to another in the fundus gland. Basically their structure is the same, however, a membrane about 60 Å in thickness along which one perceives a varying number of maximally dense particles with a diameter of some 130 Å. Moreover, free such particles are encountered scattered in the cytoplasm. The zymogen cells are the epithelial cell type in the fundus gland in which α-cytomembranes occur most abundantly. The mucoid cells contain a large number of free cytoplasmic particles whilst their α-cytomembranes are very scarce. In both parietal cells and argyrophil cells there are but a few α-cytomembranes and cytoplasmic particles. These findings agree well with previous reports concerning the epithelial cells of the fundus gland (e.g. *74*, *109*, *115*, *187*).

Interconnections between the cisternae bounded by the smooth surfaces of the α-cytomembranes have been reported in a number of cells (e.g. *2*, *20*, *49*, *93*, *104*, *105*, *175*, *213*), whereby a continuous system is found for which Palade's term "endoplasmic reticulum" (*153*, *155*) seems appropriate. In certain types of cell in other tissues interconnections allegedly exist between the α-cytomembranes and the plasma membrane (*56*, *104*, *189*). Although no interconnections of these kinds were seen in any of the epithelial cells of the fundus gland, it cannot be ruled out that they might exist although in negligible numbers.

The chemical composition of the α-cytomembranes and the cytoplasmic particles has been extensively investigated (for a review see e.g. *25*). Thus, from homogenates of a number of tissues a fraction has been isolated which is mainly composed of particles

of the same size and shape as those found in the cytoplasm of most cells. This fraction contains a very high proportion of ribonucleic acid (RNA) in the form of a protein (ribonucleoprotein, RNP). This is the reason why the maximally dense cytoplasmic particles often are designated as ribosomes.

In this light it seems highly probable that similar cytoplasmic particles in the secretory cells of the fundus glands contain RNA. This view is borne out by the fact that the RNA content in the zymogen cells is three to four times higher than in the parietal cells (*21*, *22*, *208*), an observation which seems well compatible with the relative proportions of small, cytoplasmic particles in the respective cells.

It should be pointed out in this connection that the small particles in the cytoplasm probably are artefacts or precipitation products, which arise when the intracellular milieu is altered, e.g. in conjunction with fixation or homogenization (*81*).

A succession of workers have made observations suggesting that RNA participates in cellular protein synthesis (cf. *15*, *21*). The zymogen cells and the mucoid cells of the fundus gland have a high content of RNP particles. Of old these cells have been considered to have a high protein production. Conversely, merely a few RNP particles are to be found in the parietal cells, which could mean that these synthesize little protein. The latter inference is supported by the current view that the parietal cells elaborate hydrochloric acid. According to an early hypothesis, the secretory product of the parietal cells was a protein hydrochloride which, after being released from the cell, decomposed into protein and hydrochloric acid (*82*, *90*, *214*). Not only have strong objections been raised against this theory (cf. *31*, *41*, *91*) but the low RNA content of parietal cells also suggests that their secretory products do not include proteins.

*Pigment bodies*

Formations having some resemblance to the pigment bodies encountered in the argyrophil cells have previously been observed in cells of the adrenal cortex of the mouse (*211*), in a fraction of human spinal-cord homogenate (*11*, *211*), and in the chromaffin cells of cyclostome hearts (*12*). In the case of the cyclostome hearts it was suggested that these formations were created through the fusion of secretory granule material (*12*). It cannot be ruled out that a similar mechanism is involved when the pigment bodies of the argyrophil cells of the fundus glands arise.

The pigment bodies of the adrenal cortex and of the spinal cord reportedly emitted a yellowish fluorescence when ultraviolet irradiated and probably belonged to the group of lipofuchsin granules (*11*, *211*), the lipofuchsins being related to the so-called *Abnützungspigment*. Using histochemical methods, Feyrter (*65*) could demonstrate in argyrophil cells of gastro-intestinal carcinoid tumours the existence of a substance which was at least similar to the *Abnützungspigment*. Under ultra-

violet irradiation the argyrophil cells of the fundus gland also emit a yellowish fluorescence that Hamperl (*79*) considered characteristic of lipofuchsins. It should be mentioned, however, that a yellow fluorescence (also noted by the present author in formalin fixed, deparaffined sections from the gastric mucosa of the mouse) can also be emitted by the secretory granules of the argyrophil fundus-gland cells.

In the light of these observations it can be postulated, that what is here called the pigment bodies of the argyrophil cells are comparable to the pigment bodies of other tissues and are the submicroscopic equivalent to lipofuchsin granules.

*Dense microbodies and lipid droplets*

So-called dense microbodies have been encountered in all types of exocrine cells in the fundus glands. Similar organelles have previously been observed in a variety of other cells (*45*, *145*). From liver, among other tissues, de Duve (*45*) was able to prepare a fraction containing a high proportion of such organelles, which he called lysosomes. He described them as round, solid formations occasionally exhibiting less dense regions; they were provided with a surface membrane and had a mean diameter of 0.4 $\mu$. Biochemical analysis of this fraction disclosed a high content of several enzymes with an acid pH optimum. However, since the fraction apparently to some extent was contaminated with mitochondria it cannot entirely be ruled out that some of the enzymes demonstrated were of mitochondrial origin.

Apart from the fact that the dense microbodies occurring in the fundus gland generally lack a distinct surface membrane, their appearance agrees on the whole with that of the so-called lysosomes.

In a number of different types of cell certain formations have been found which have been considered to be lipid droplets (cf. *73*, *211*). The ultrastructure of these formations is almost identical with that of the "lipid droplets" in the present investigation. Furthermore, with the aid of the light microscope, structures supposed to be lipid droplets have been found in some of the cells of the gastric mucosa (*147*). Consequently it seems justifiable to use the term lipid droplets to designate the structure in question.

*Nuclei*

The nucleus is surrounded by a three-layered surface membrane which in most cells, including the fundus-gland cells, is provided with discontinuities. A numbe of workers have demonstrated that such discontinuities sometimes have a thin covering membrane (e.g. *1*, *206*). Membranes of this kind have occasionally been seen in the present investigation, too. However, such covering membranes have been considered an illusion due to projection across the discontinuities of portions of the surrounding nuclear membrane which have been included in the sections (*6*, *207*).

In a number of different cells another type of communication has been observed between the nucleus and the cytoplasm. The less dense layer of the nuclear membrane then communicates with the cisternae bounded by α-cytomembranes (*17*, *49*, *56*, *74*, *93*, *129*, *152*, *160*, *162*, *171*, *176*, *177*, *206*, *207*), the outermost nuclear membrane layer merging with an α-cytomembrane. Accordingly it has been postulated that the nuclear membrane is a specially adapted α-cytomembrane pair (*153*, *156*, *206*, *207*, *210*). Even though such communications were not encountered by the present author in the epithelial cells of the fundus gland, the possibility cannot be ruled out that they may exist, if only as rather rare transient formations.

## Comparisons between Methacrylate Embedded Tissues Fixed at pH 7.2 and at pH 8.5

Previously, ultrastructural differences between methacrylate embedded cells fixed at various pH have been studied by Palade (*150*, *152*) in liver, Rhodin (*168*) in kidney and Zetterqvist (*213*) in jejunum, all three achieving optimal tissue preservation at or near pH 7.2. Whereas Palade observed manifestations of poor preservation after fixation in acid and alkaline fluids, Rhodin and Zetterquist reported such manifestations only after fixation in acid fluids and not in tissue fixed at more alkaline pH levels. In previous studies on the ultrastructure of the gastric mucosa the pH of the fixation fluid has ranged from 7.1 to 7.8.

For the present investigation the pH of the fixation fluid was 8.5, the highest alkalinity compatible with high capacity of the adopted buffer system. An alkaline fixation fluid was employed to reduce proteolytic changes due to the pepsin secreted by the gastric mucosa, the factor deemed mainly responsible for the excessive proportion of badly preserved cells in fundus glands fixed at pH 7.2. Calcium chloride was added for its imputed ability to promote penetration of the embedding medium (*97*) and to improve the preservation of the tissue (*152*, *163*). However, no differences could be observed in penetration of the embedding agent when calcium chloride was omitted from the fixation fluid, nor in quality of preservation. Similarly, no major changes in the shape or in the dimension of the cytoplasmic structures seem to occur when the sections were afterstained.

On examining gastric mucosa from the corpus region fixed at pH 8.5 one is immediately struck by the fact that most of its cells seem well preserved. For in such tissue fixed at pH 7.2 the reverse is the case: the majority of cells of all types are badly preserved, and especially the mucoid cells are very seldom even reasonably well preserved.

Compared with tissue fixed at pH 7.2, the outstanding ultrastructural deviation in cells after fixation at pH 8.5 is the consistently higher density of the cytoplasmic ground substance, for example around the vacuoles of parietal cells or between the α-cytomembranes of zymogen cells. Whereas here the cytoplasmic ground substance usually is of low to moderate density after fixation at pH 7.2, it is usually of appreciably higher density in cells fixed at pH 8.5. Otherwise the use of an alkaline fixation fluid was associated with only a single ultrastructural characteristic in well preserved cells. Thus the zymogen granules within the same cell then exhibited a uniform, in most cases lower density than those of zymogen cells fixed at pH 7.2 where the density of the zymogen granules varied greatly within the same cell. It is interesting to speculate upon what might cause this change in pattern.

First, however, it will be necessary to consider some possible reasons for the density differences between *zymogen granules after fixation at pH 7.2*. The moderately dense appearance of some zymogen granules could be due to mucin content, an explanation used for the moderate density of some of the secretory granules in the acinar cells of the lacrimal gland (*181*). Although Bowie's finding (cited from Babkin, *3*) that zymogen cells respond to some stains for mucus lends support to such a hypothesis, it is difficult to reconcile with Menzies' (*127*) statement that the mucinogen content of the granules in zymogen cells vanishes at an early stage of the neonatal development of the mouse.

The varying density of the zymogen granules could also reflect differences in their maturity in the way suggested for the secretory granules in epithelial cells of some other glands (*135*, *138*, *178*, *181*). Accordingly, the small zymogen granules found in the vicinity of the Golgi apparatus might represent immature granules, the larger dense ones somewhat more mature granules, and the large ones of moderate density the most mature granules. The zymogen granules of moderate density in the apical part of the cell might represent granules releasing their content into the lumen (Figs. 3, 7) by a mechanism observed in the acinar pancreatic cells (*55*, *157*). Another releasing mechanism, suggested for the acinar and intercalary duct cells of the parotid gland (*178*), consists of dissolution of the granular content prior to its release to the lumen. Such a dissolution process could explain the fragmentation of the surface membrane of the zymogen granules and the loss of their dense content.

Whatever might be the causes contributing to the varying density of the zymogen granules, a dominant factor in the creation of the moderately dense granules would seem to be postmortal changes. The present investigation disclosed that in methacrylate embedded zymogen cells fixed at pH 7.2 the dense type of zymogen granules was absent in poorly preserved cells. Most probably the presence of pepsin in an acid environment had given rise to proteolytic changes in the gastric mucosa, the zymogen granules being affected as well. Similarly, Leduc *et al.* (*116*) found that the zymogen

granules in the acinar cells of pancreatic tissue fixed in formalin and embedded in a water-soluble polyepoxide became markedly less dense after brief exposure to the action of pepsin in an acid environment.

The low density of the zymogen granules in most of the *zymogen cells fixed at pH 8.5* can hardly be a sign of poor preservation owing to proteolytic action, for then the other organelles would certainly show similar signs. Obviously the density of zymogen granules in the electron micrograph is more or less proportional to their content of reduced osmium tetroxide. Logically, therefore, the alkaline milieu could have reduced the osmiophilia of the substance in the granules or, alternatively, rendered it more soluble.

Summing up, it can be said that the use of an alkaline fixation fluid makes the epithelial cells of the fundus gland clearly better preserved than after fixation at pH 7.2. However, the usage of fixation fluids with different pH is attended by certain differences in the ultrastructural appearance of some cellular organelles and in the cytoplasmic ground substance. Still, from morphological point of view, the designation poor preservation does not seem appropriate in most of these cases. It rather seems that different components of the respective organelles will be preserved when fixation fluids of different pH are used. It appears highly probable that any type of cell at any degree of functional activity requires a special pH range of the fixation fluid to become morphologically well preserved. Furthermore, variation within this pH range will probably exert a profound influence on what chemical components of the respective organelles will be preserved, and to which extent.

## Comparisons between Methacrylate and Vestopal Embedded Tissues

Zelickson *et al.* (*212*) emphasized that the most striking difference between methacrylate and Vestopal embedded epidermal tissue was the clarity of the internal cellular structure of the tissues embedded in the Vestopal. The endoplasmic reticulum, the mitochondria, the nuclear and plasma membranes, and the desmosomes with their tonofilaments stood out sharply but the general contrast was poorer than in the methacrylate embedded sections. Condie *et al.* (*30*) examined the ultrastructure of myelin sheaths embedded in different media. They found that the tissue was considerably less distorted when it was embedded in Vestopal than in methacrylate or araldite. Hitherto nobody else seems to have used Vestopal as embedding medium for the gastric mucosa.

On comparing the ultrastructure of methacrylate embedded and Vestopal embedded fundus glands one is immediately struck by the high density of the latter embedding medium, a property mentioned by several previous workers (e.g. *166*). Indeed, the

high density of Vestopal would seem to furnish at least a partial explanation for the lack of nuances in the density of the ground cytoplasm and the matrix of the organelles.

All membranous structures are visualized much more distinctly in Vestopal sections than in methacrylate sections. A far higher proportion of membranes is depicted as being composed of three layers. Occasionally indeed, it has appeared that the dense layers of the mitochondrial membranes are themselves made up of three lamellae each having a thickness of the order of 15 Å. Similarly structured, dense mitochondrial membranes layers have previously been observed in a number of other cells (*2*, *20*, *68*, *168*) in methacrylate embedded tissues.

The surface membrane of zymogen granules likewise often appears composed of three layers. The observations made here do not verify Lillibridge's (*121*) contention that only small zymogen granules (less than 1 $\mu$ in diameter) have a three-layered surface membrane whilst large zymogen granules have a monolayer surface membrane.

The present investigation provides no clues to the reason why membranous structures are more distinctly visualized in Vestopal sections than in methacrylate sections. However, previous workers have attributed this phenomenon to unequal rates of sublimation from the surface of the sections. Thus, Reimer (*167*) showed that whereas irradiation in an electron microscope reduced the mass of a Vestopal section by 10–15%, the corresponding figure for a methacrylate section could be as high as 55%. The diminution in mass of the methacrylate sections was caused partly by evaporation from the surface of the sections, partly by internal chemical transformation with loss of non-carbonic constituents. Surface evaporation probably contributed insignificantly to the reduction in mass of the Vestopal sections, which has also been demonstrated after shadow-casting irradiated sections (*167*, *179*). Furthermore, differences in the degree of dissolution of tissue substance during dehydration and infiltration might affect the distinctness whereby membranes are delineated.

Some of the structures in Vestopal embedded tissue exhibit significantly larger dimensions than the corresponding structures in methacrylate. This is probably so because the two embedding media shrink unequally during polymerization. Thus, whereas polymerization of Vestopal embedded tissue is attended by a shrinkage of between 7 and 10% (*166*) the corresponding figure for methacrylate embedded tissue is of the order of 20% (*4*). Although dehydration causes a considerable shrinkage of the tissue, no differences seem to exist in this respect between alcohol and acetone (*30*).

Except for alteration of volume, cellular organelles of the same type embedded in the different media rarely exhibit any differences in their ultrastructure. The vacuoles in the parietal cells are an exception however. Whilst they have the same

mean diameter in the two embedding media, their outline tends to be smoothly rounded in methacrylate sections but not seldom somewhat irregular in Vestopal sections. These structural differences could also be due to unequal shrinkage.

One might include among structural differences the fairly frequent finding that Vestopal has not penetrated into some of the membrane-bound regions. Such imperfect penetration, never encountered in methacrylate sections, might depend on the high molecular weight of Vestopal. Another possibility is that the tissue may contain liquid or gas which becomes trapped locally. However, the presence of such "bubbles" has little effect upon the preservation of the tissue as a whole.

Studying post-mortal changes in liver mitochondria and nuclei, David (*40*) found that in Vestopal sections these changes, predominantly swelling of the mitochondria with loss of their inner membranes, and vacuolization and granulation of the karyoplasm, had the same appearance as, but were far less pronounced than, those in methacrylate sections. In the present investigation such alterations of the ultrastructure were only exceptionally encountered in Vestopal embedded fundus glands. David attributed the absence of more conspicuous signs of poor preservation in Vestopal sections and the presence thereof in methacrylate sections of the same tissue to different modes of polymerization—whereas the polymerization of Vestopal was gentle to the tissues, that of methacrylate exposed them to destructive forces. Differences in quality of preservation between gastric mucosa embedded in Vestopal and methacrylate could also be thus explained.

To sum up, it has been shown that Vestopal possesses both advantages and disadvantages compared with methacrylate as an electron microscopic embedding medium for the gastric mucosa. The advantages over methacrylate are the better chances of securing adequate tissue preservation, the more distinct visualization of membranes, and the lesser degree of shrinkage. The disadvantages of Vestopal are its high electron density and its inability to permeate the tissue completely. Furthermore, Vestopal blocks are more difficult to section than methacrylate blocks.

## Cell Identification

### *Zymogen cells*

The outstanding light microscopic characteristics of the zymogen cells are the abundance of zymogen granules in the supranuclear and paranuclear cytoplasm and the pronounced basophilia of the infranuclear cytoplasm, a property ascribed to the high local RNA concentration (*208*). This cell consequently assumes a close light microscopic resemblance to the acinar cells of the pancreas. The light microscopic appearance of zymogen cells agrees well with the electron microscopic visualization of that cell which the author has designated as the zymogen cell of the fundus

gland. The latter does not only exhibit an accumulation of granules in the supranuclear and paranuclear portions of the cytoplasm as well as an abundance of RNP particles basally, but its ultrastructural similarities to the pancreatic acinar cell (cf. *196*) are also striking. It will suffice to mention that the two cell types in many cases have very similar appearance of the α-cytomembranes, Golgi apparatus, and zymogen granules.

Furthermore, none of the other epithelial cells of the fundus gland exhibits an ultrastructural organization which is anywhere near as compatible with the light microscopic appearance of the zymogen cell as what is here called the zymogen cell. Consequently the author feels justified in declaring that this cell is identical with the zymogen cell in light microscopy.

*Parietal cells*

The intracellular canaliculi have been a distinguishing feature of the parietal cell ever since the days of Golgi. The numerous mitochondria in the otherwise fairly nondescript cytoplasm were also observed by early light microscopists. In the author's opinion the occurrence of mitochondria in large numbers and of intracellular canaliculi in the electron microscopic image of one epithelial cell type of the fundus gland constitutes very strong evidence of its being identical with the light microscopist's parietal cell. Without exception, all those who previously have studied the ultrastructure of the parietal cell share this view.

*Mucoid cells*

Those cells which here have been called mucoid cells constitute a somewhat heterogeneous group from the point of view of their ultrastructural appearance. The cells vary in shape and in the frequencies of several intracellular organelles. Nevertheless they possess certain characteristics which justify their classification as one and the same type of cell. Thus, they invariably possess a more or less undulated plasma membrane along their lateral surfaces and large intra-mitochondrial membrane-bound granules. At the same time these are features whereby the mucoid cells can be distinguished from the other epithelial cells of the fundus glands. Among other ultrastructural details contrasting these cells from other fundus-gland cells it will suffice to mention the diameter of the secretory granules, which is only about one-half of that of the zymogen granules, the scarcity of α-cytomembranes, and the abundance of free RNP particles.

Also from a light microscopic point of view the resemblance has been stressed between the mucoid cells of the fundus glands, i.e. between the surface epithelial cells and the mucous neck cells (*180*). Moreover, gradual changes between these two types of cell are said to occur (*8*, *9*, *64*, *94*, *95*).

The identification of what the author has called mucoid cells with the mucoid cells of the light microscopists is supported by strong evidence. The distribution of the secretory granules—namely, a concentration to the zone immediately beneath the apical cell surface—is perhaps the most outstanding point. The identification has been proved in those cases where the author has examined cells at the surface of the gastric mucosa, i.e. surface epithelial cells according to light microscopists. The ultrastructure of these cells essentially agrees with that of others belonging to the cells defined as mucoid by the present author. Finally, the identification is borne out by certain ultrastructural similarities to mucoid cells in other regions of the gastro-intestinal tract (*134*).

*Argyrophil cells*

In the main the identification of the argyrophil cells is based on striking ultrastructural similarities to other cells which are classified as argyrophil on account of their histochemical properties (*80*), for example the chromaffin cells of the adrenal medulla (*47*), the α-cells of the pancreatic islets (*110*, *139*), the glomus cells of the carotid body (*118*), and the chromaffin cells in cyclostome hearts (*12*). These similarities are in the first place concerned with the appearance, number, and distribution of the granules but the other intracellular organelles are also of similar appearance in the cell types listed above.

Further evidence is the position of the cells. When studied light microscopically the argyrophil cells lack contact with the lumen of the fundus glands (cf. *27*). In the present study the same applies only to those cells which have been called the argyrophil cells.

# PART II. REFED MICE

## CHAPTER 5

## Survey of the Literature, Materials and Methods

### ABSTRACT OF LIGHT MICROSCOPIC OBSERVATIONS

The light microscopic appearances before and after stimulation of the epithelial cells of the fundus glands have been compared in several mammalian species including the mouse. The modes of stimulation have varied, but in so far as stimulation really has been induced, the changes in the corresponding cell types from different species have been similar.

The alterations which start when the gastric secretion is stimulated, can progress for many hours. Thus it has been shown that more than 18 hours after refeeding some cells in the fundus glands still exhibit deviations from their light microscopic fasting appearance (e.g. *208*). The following brief review includes merely the more important alterations observed during the first few hours after stimulation.

In the *zymogen cells* the amount of zymogen granules decreases (*14*, *71*, *113*, *123*, *208*), at the same time as the amount of osmiophilic substance in the cytoplasm increases (*71*, *137*). This substance has in light microscopy been considered to be the Golgi apparatus. Moreover, using other staining methods, an increase in the extent of the Golgi apparatus has been postulated (*137*). Furthermore the mitochondria, which are described as granular in the fasted mice, become longer and more numerous during the secretory process (*71*, *124*). The extent of the "ergastoplasm" in the basal part of the cytoplasm increases (*123*), and it has also been shown that the amount of cytoplasmic RNA rises (*208*). The nucleus is said to grow larger (*123*), and its surface membrane becomes thicker (*208*).

In the *parietal cells* the modification of the appearance is less conspicuous. The mitochondria become fewer (*71*, *123*) and tend to collect along the lateral and basal cell surface. The diameter of the intracellular canaliculi increases (*72*, *90*, *111*). Moreover, it has been found that the succinic dehydrogenase content of the parietal cell is augmented (*184*, *205*). Otherwise no appreciable changes seem to occur.

After prolonged stimulation the amount of secretory granules in the *mucoid cells* is reduced (*8*, *101*). However, the extent of this reduction varies greatly between different mucoid cells (*101*).

No change in the appearance of the *argyrophil cell* seems to have been reported following stimulation of gastric juice production.

## ELECTRON MICROSCOPIC LITERATURE

A number of investigations have been made on the changes in the ultrastructure of the epithelial cells of the mammalian fundus glands after stimulation. In some cases food has been given (*115*, *184*, *191*), in other cases histamine (*34*, *184*, *185*, *187*, *204*), insulin (*184*, *187*) or pilocarpine (*75*, *78*) has been injected in order to stimulate the gastric secretory activity. Such a stimulation has also been attained by stimulating the vagus nerves electrically (*185–187*). Regardless of which of the above ways of stimulation has been used, the observed ultrastructural changes are on the whole the same.

### *Parietal cells*

The following ultrastructural changes have been reported in parietal cells after stimulation. Concentrating in the neighborhood of the intracellular canaliculi (*78*, *184–187*) the vacuoles become fewer (*78*, *184–187*, *191*, *204*). However, this decrease in the number of vacuoles is said to be preceded by an increase in their number (*184*, *185*). Occasional vacuoles are reported to communicate with the glandular or canalicular lumen (*78*, *186*, *187*). The intracellular canaliculi seem to become more extensive (*187*, *204*) and dilated (*78*, *191*), and their surface area is enlarged by the development of large numbers of closely approximated microvilli (*187*). A constriction of some of the intracellular canaliculi has also been reported (*184*, *186*, *187*). The mitochondria are said to exhibit an increase in their diameter (*186*, *187*), and in the distance between their inner membranes (*186*). Furthermore the density is reduced in the whole (*186*) or in some parts (*187*) of the mitochondrial matrix. The cytoplasmic particles become more abundant (*184*, *185*, *191*). The increase in size (*75*, *78*) and number (*75*) of the vacuole-containing bodies reported by one author has not been confirmed by others (*187*, *204*).

### *Other epithelial cells*

Only one investigation has been performed on the ultrastructural changes in the other epithelial cells of the fundus glands (*108*, *191*). In this case rats were examined which had been stimulated by food administration. The results may be summed up

as follows. In *the zymogen cells* the number of zymogen granules is markedly reduced. The Golgi vacuoles and vesicles become larger, and in their neighborhood small, rather dense zymogen granules may occur. The larger the zymogen granules, the lower their density, and the more gaps appear in their previously intact surface membranes. The mitochondria migrate to the vicinity of the Golgi apparatus and often abut against secretory granules. The long strands of $\alpha$-cytomembranes are broken up into portions bounding cisternae of varying size and shape containing a more or less dense substance. In the *surface epithelial cells* stimulation is attended by enlargement of the Golgi vesicles, and in the *argyrophil cells* no ultrastructural changes are apparent. Concerning the *mucous neck cells* see p. 13.

## MATERIALS AND METHODS

This study was made on 8 female albino mice belonging to one of the strains used in Part One. After a 36 hours' starvation period under the same conditions as described in Part One (p. 13), the mice were let out into a glass jar where they had free access to water and food. This way of stimulating the gastric juice production was chosen, since it gives rise to an all-round, physiological activity of the cells. The changes occurring at an early stage of digestion were considered most interesting to study initially. Consequently the mice were sacrificed 30 minutes after they were put into the glass jar. The stomachs of the mice then contained plenty of food. In the following they were treated exactly as set out in Part One. Thus fixation took place at a pH of 7.2 or 8.5, and the tissue blocks were embedded either in methacrylate or in Vestopal. However, since examination of sample sections from these tissue blocks disclosed that only those fixed at pH 8.5 and embedded in Vestopal W were adequately preserved, the observations on the morphology were made solely on tissue treated in this way.

## CHAPTER 6

# Results

Only those formations which differed in structure and/or dimensions from the corresponding formations in specimens from unstimulated animals will be considered. Changes occurring in the number of cellular organelles cannot be exactly calculated when using the electron microscopic methods adopted in the present investigation. Thus, when alterations in the number of certain organelles are said to occur, this information is based on estimations made on a large number of sections.

## ULTRASTRUCTURAL ORGANIZATION OF FUNDUS GLANDS

The organization of the fundus glands exhibits no major poststimulatory changes. The sole appreciable alteration seen affects the lumen of the gland. Having a diameter of a few microns in the starved animals, the lumen is often collapsed in the stimulated mice and takes on the appearance of a narrow slot (Fig. 39).

## ULTRASTRUCTURE OF THE SECRETORY CELLS OF THE FUNDUS GLANDS

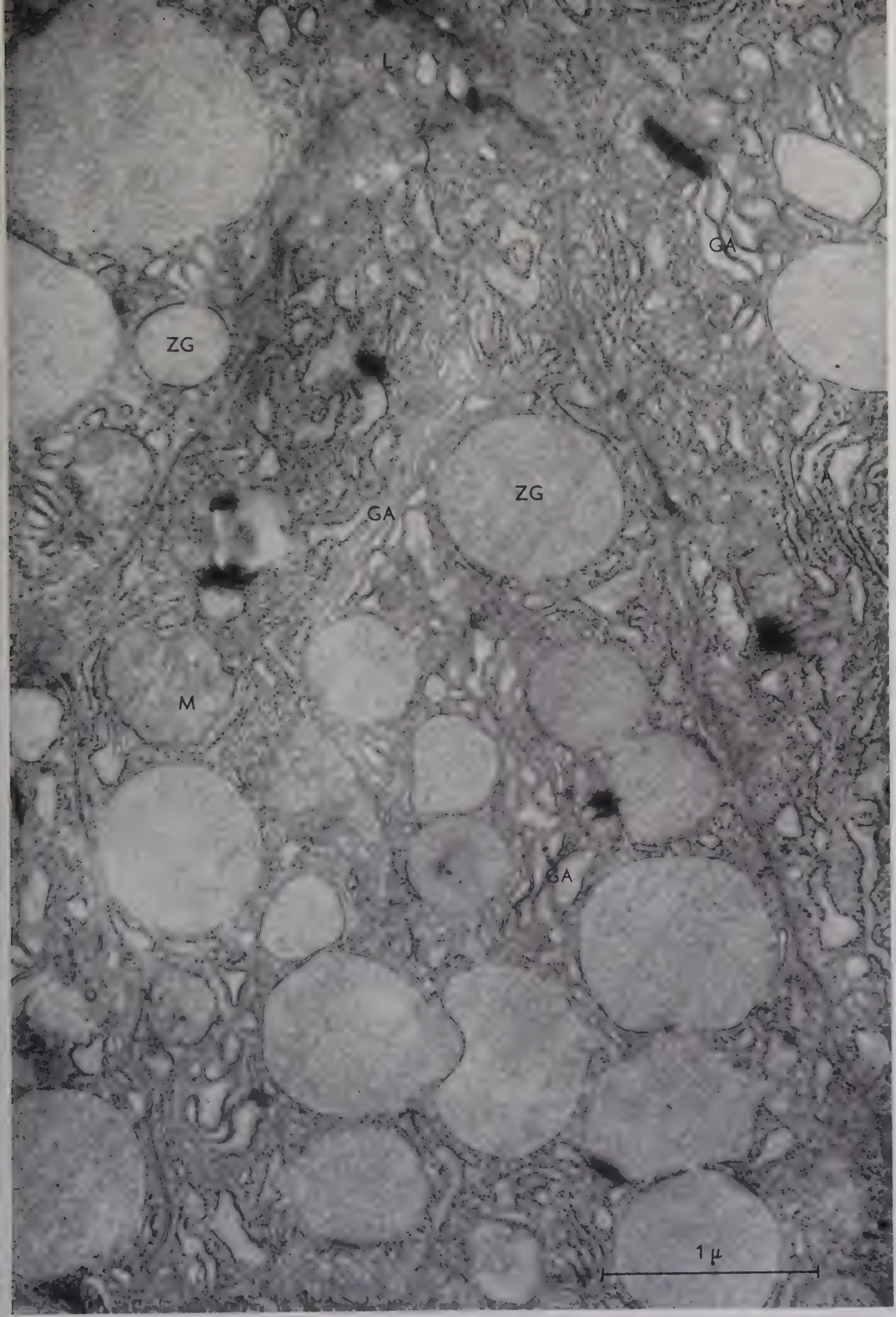
The degree of the poststimulatory ultrastructural changes observed in the epithelial cells of the fundus gland varies with the type of cell. The changes visualized in the electron microscope after refeeding may even vary considerably from one cell to another of the same type. Only consistently occurring changes will be described in the following.

*Zymogen cells*

After refeeding, one observes in the zymogen cells a significant diminution of the mean diameter of *zymogen granules* to 0.75 from about 1.04 $\mu$ in the starved controls.

---

FIG. 39. Apical portions of zymogen cells from a refed mouse. Closely spaced microvilli nearly occlude the glandular lumen (*L*). Unlike the case of fasted mice, the Golgi apparatus (*GA*) are not confined to a limited cytoplasmic region but occupy extensive areas in many parts of the cytoplasm. Golgi structures very often lie between the numerous, comparatively small zymogen granules (*ZG*). The $\alpha$-cytomembranes (*A*) bound cisternae of varying width, though as a rule wider than in fasted mice. The ultrastructure of the mitochondria (*M*) does not seem to exhibit postprandial changes. Vestopal embedded section fixed at pH 8.5. $\times$36,000.


L
GA
ZG
ZG
GA
A
M
GA
1 µ

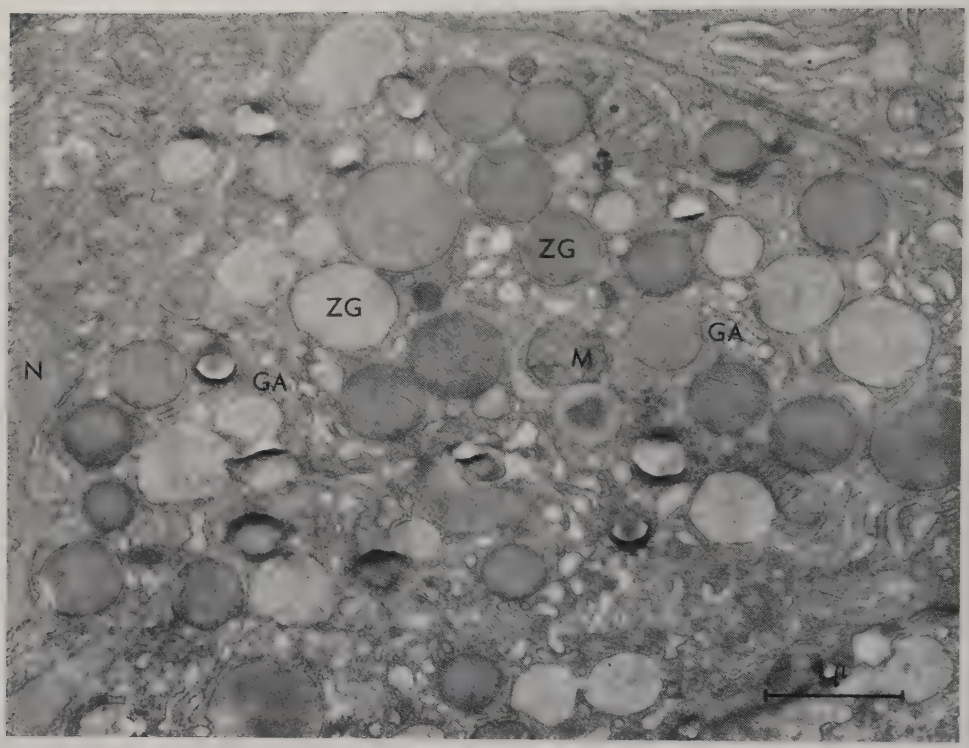
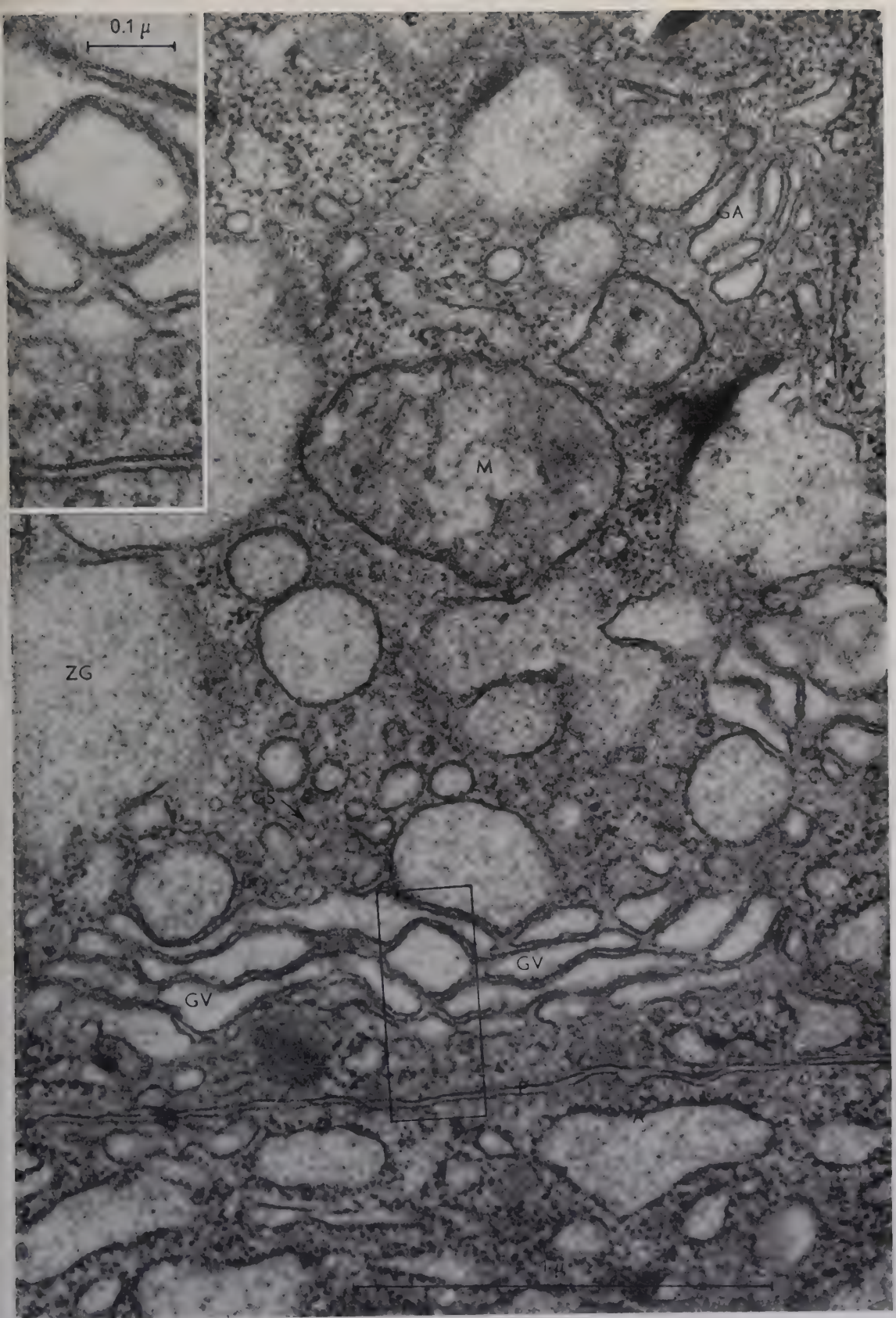



FIG. 40. Part of a zymogen cell from a refed mouse. Golgi structures (*GA*) occur in many parts of the cytoplasm, which also contain numerous zymogen granules (*ZG*) of varying density. Some of the zymogen granules have a core of another density than the rest of the granule. Sometimes similar zymogen granules occur in fasted mice also. *M*, mitochondrion: *N*, nucleus. Vestopal embedded section fixed at pH 8.5. ×18,000.

FIG. 41. Detail of a zymogen cell from a refed mouse, featuring a large number of Golgi structures (*GA*, *GV*, *GS*). The Golgi vacuoles (*GV*) seem larger and the Golgi vesicles (*GS*) much more abundant than in fasted mice. There are several zymogen granules (*ZG*) of varying size, the smaller being hard to distinguish from Golgi vacuoles or Golgi vesicles. The cisternae bounded by α-cytomembranes (*A*) are wider than in fasted mice. *M*, mitochondrion; *P*, plasma membrane. The inset shows in greater detail the area in the rectangle. The three layers composing individual Golgi and plasma membranes are now evident. Similar appearances may also be observed in fasted mice. Vestopal embedded section fixed at pH 8.5. ×71,000; inset, ×150,000.


0.1 μ
GA
M
ZG
GS
GV
GV
A

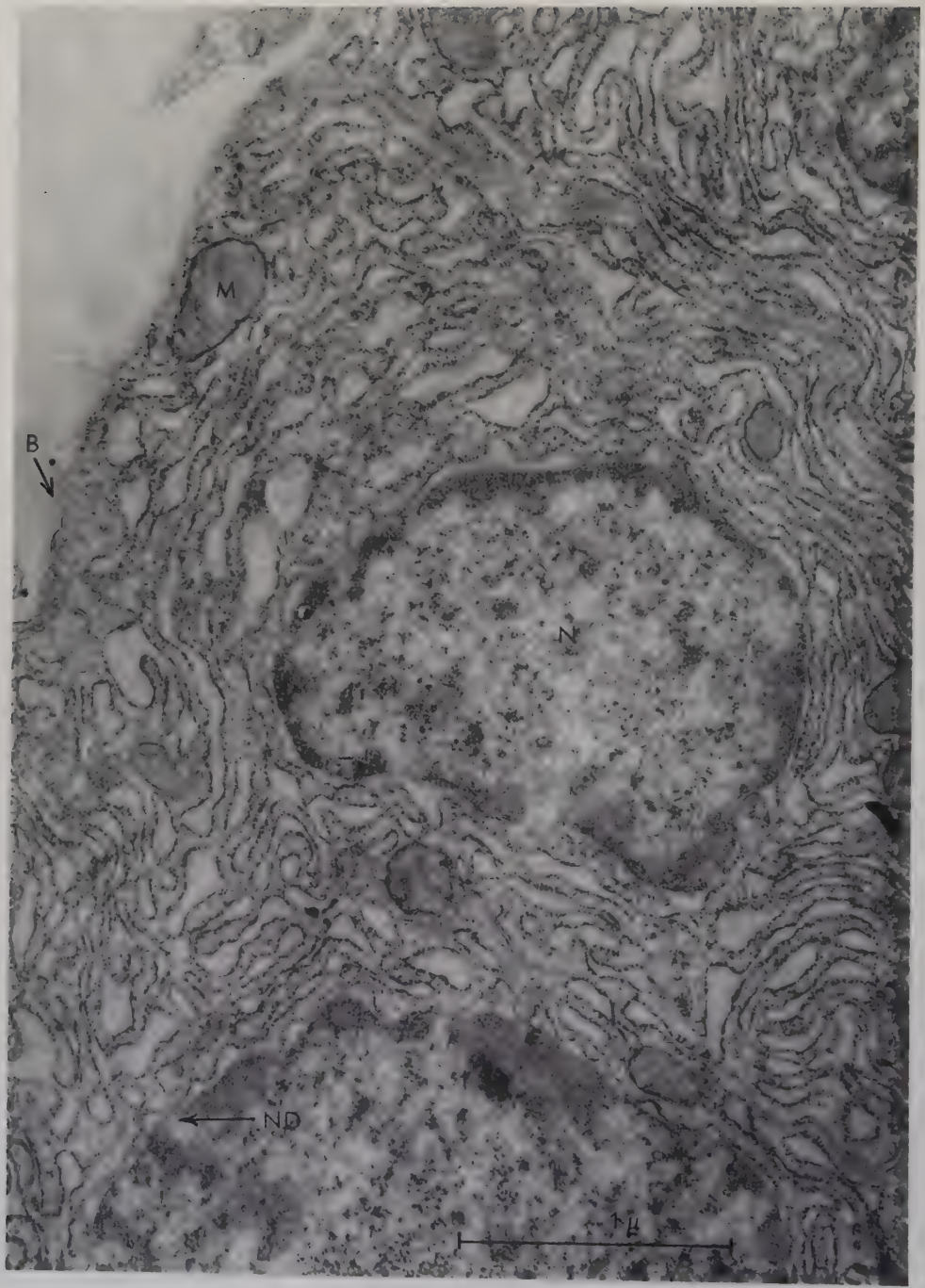



FIG. 42. Basal portions of zymogen cells from a refed mouse. The α-cytomembranes—disposed much less regularly than in fasted mice (cf. Fig. 5)—enclose cisternae of varying width. The nuclei (*N*) have an irregular outline, and the less dense layer of the nuclear membrane exhibits expansions of varying appearance. Some discontinuities of the nuclear membrane (*ND*) are also visible. *M*, mitochondrion; *B*, basement membrane. Vestopal embedded section fixed at pH 8.5. × 37,000.

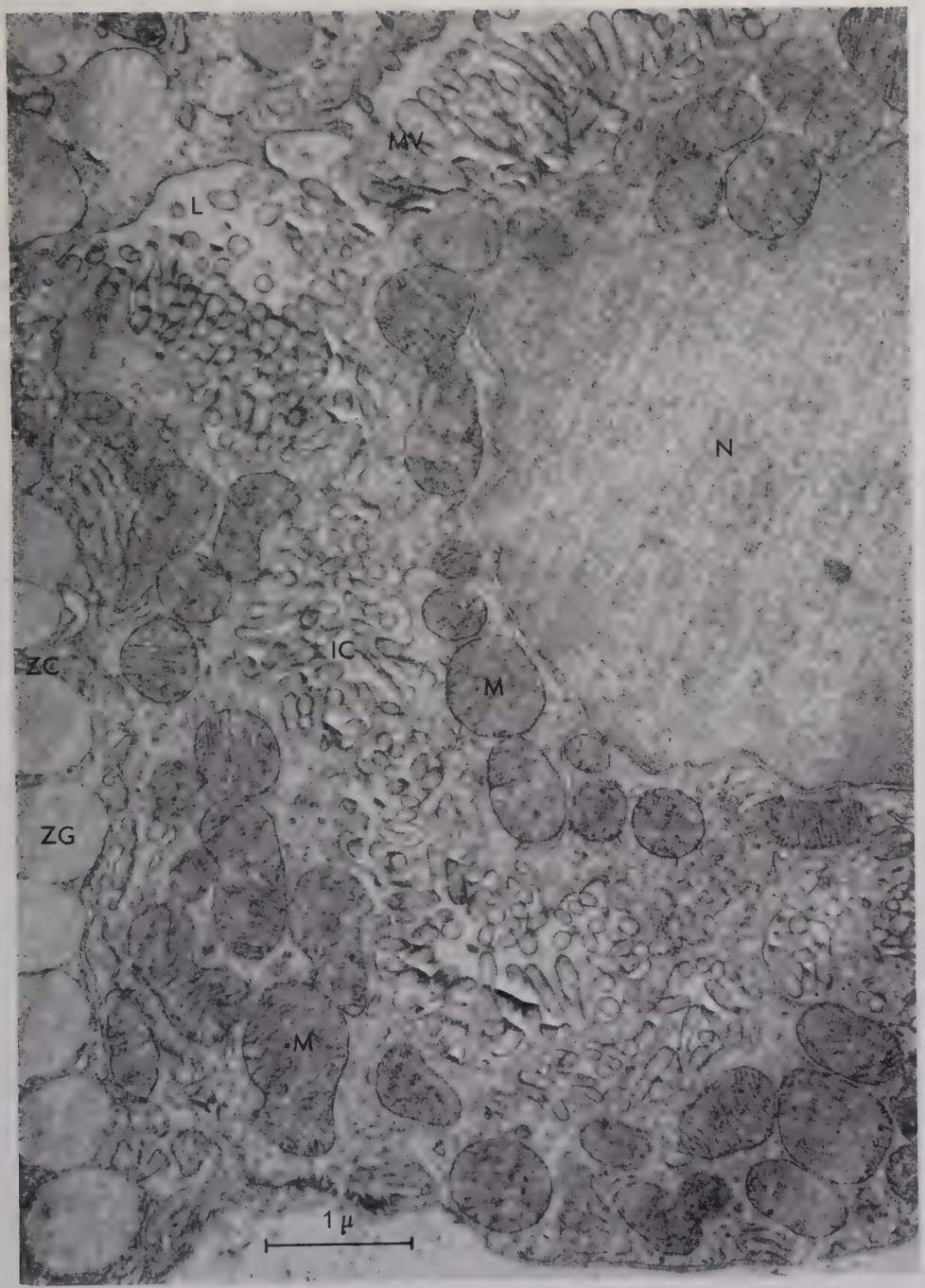



FIG. 43. Survey of a parietal cell from a refed mouse. Microvilli (*MV*), many of which have a club-like shape, project from the apical cell surface into the glandular lumen (*L*) and are also abundant in the intracellular canaliculus (*IC*). The cytoplasm contains mitochondria (*M*) in great numbers but almost no vacuoles. To the left is part of a zymogen cell (*ZC*) with zymogen granules (*ZG*). *N*, nucleus. Vestopal embedded section fixed at pH 8.5. ×20,000.

The number of zymogen granules does not seem to change. The content of a zymogen granule from a stimulated mouse has much the same appearance as in the starved mice (Figs. 39–41).

A further effect of refeeding is expansion of the cytoplasmic region occupied by the *Golgi apparatus*. The Golgi vacuoles apparently become both larger and more numerous, the configuration of most of them being irregular and of a few nearly circular. The Golgi vesicles seem more abundant than in starved mice. The Golgi vacuoles as well as the Golgi vesicles contain a substance of moderately high density, not seldom making it practically impossible to distinguish them from the numerous small zymogen granules encountered in the neighborhood of the Golgi apparatus (Figs. 39–41).

The disposition of the *α-cytomembranes* in the stimulated mice is less regular than in the starved controls. Whereas the particle-bearing α-cytomembrane surfaces are separated by a fairly constant distance of about 300–500 Å, the interspaces between the smooth surfaces are highly variable and not seldom become as wide as several tenths of a $\mu$ (Figs. 39, 41, 42). Hence the α-cytomembranes in the zymogen cells lose their regular arrangement in roughly parallel pairs which is characteristic of the starved controls (cf. Figs. 4, 5). The appearance of the content of the cisternae bounded by the smooth α-cytomembrane surfaces is similar to that in unstimulated mice.

The outline of the *nucleus* is much more irregular after stimulation than in the controls. The width of the less dense layer of the nuclear membrane varies greatly, more so than in starved mice (Fig. 42).

*Parietal cells*

*Microvilli* are encountered in much greater abundance on the apical and canalicular cell surfaces than in the starved controls; they also seem to be more irregularly shaped. Since they are often tortuous, they can rarely be followed for their entire length. Not seldom club-shaped microvilli are encountered too (Figs. 43, 44). The mean diameter of the microvilli after refeeding is some 970 Å, a figure not significantly different from the corresponding figure for unstimulated mice.

The *intracellular canaliculi* seem to have a slightly larger diameter in stimulated than in starved mice. After refeeding the canalicular lumen is often almost wholly occupied by closely packed microvilli (Figs. 43, 44).

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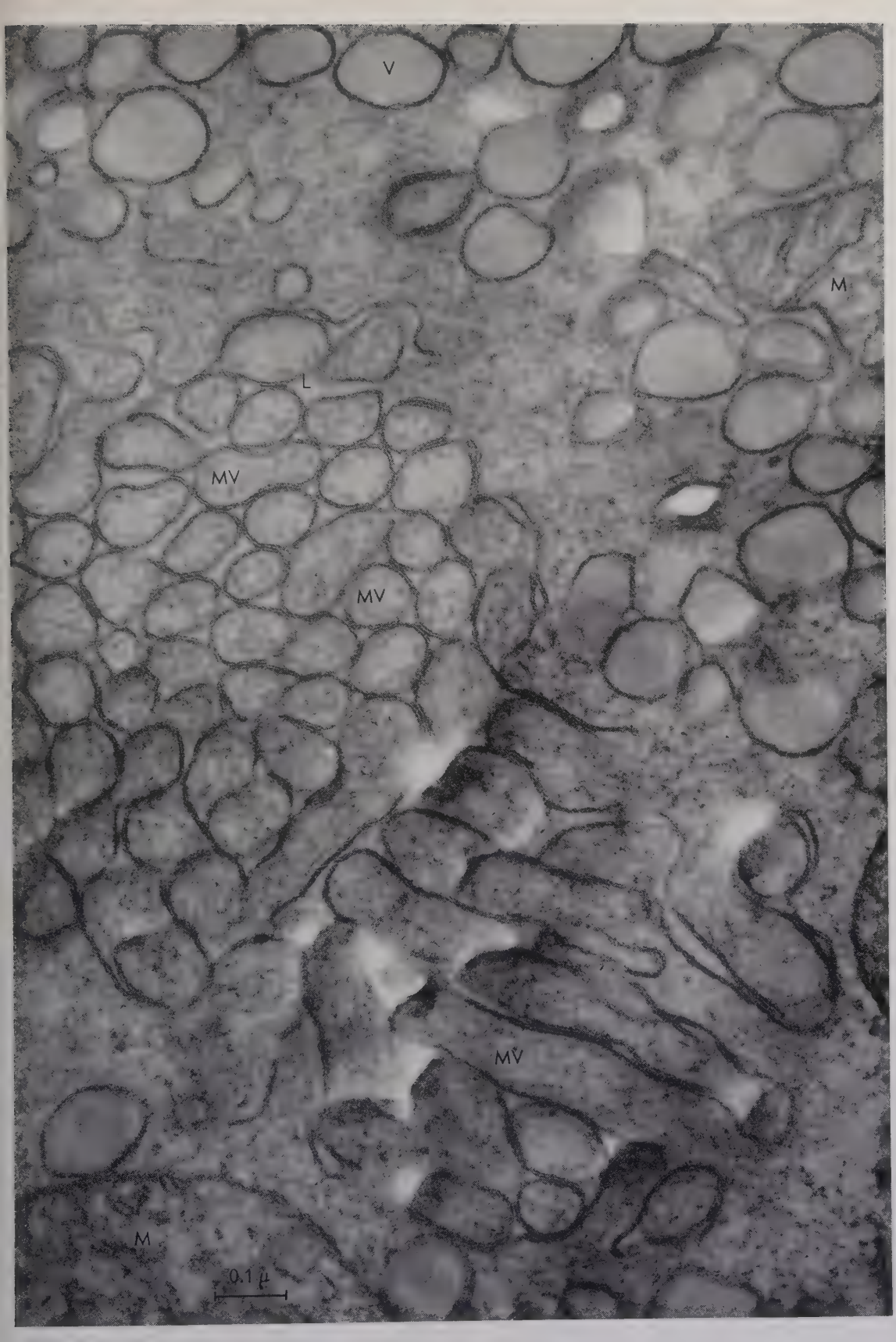
FIG. 44. Detail of part of a parietal cell from a refed mouse. The visible portion of an intracellular canaliculus has its lumen (*L*) nearly occluded by an abundance of transversely or longitudinally sectioned microvilli (*MV*). The microvilli and canalicular walls have three-layered plasma membranes. The outermost plasma membrane layers of contiguous microvilli appear to be fused. The cytoplasmic vacuoles (*V*) also have a three-layered surface membrane. The embedding medium apparently has failed to infiltrate some membrane-bound regions. *M*, mitochondrion. Vestopal embedded section fixed at pH 8.5. × 120,000.


V
M
L
MV
MV
MV
M
0.1 μ

The quantity of cytoplasmic *vacuoles* in one cell may differ markedly from that in another cell. In most parietal cells their number is markedly reduced, in a few it is essentially unchanged (Fig. 43). In both cases the distribution of vacuoles is generally the same as in the starved mice. Neither the dimensions nor the appearance distinguish the vacuoles of the stimulated animals from those of the starved controls.

*Mucoid cells*

After refeeding the mucoid cells display no consistent ultrastructural changes.

*Argyrophil cells*

No postprandial alterations have been observed in the ultrastructure of the argyrophil cells.

# CHAPTER 7

# Discussion

## COMMENTS ON MATERIALS AND METHODS

The mouse, like the rat, probably belongs to that category of animals in which a certain amount of *hydrochloric acid* is known to be produced continuously (*10*, *48*, *70*, *107*). Thus, in the present study, mice subjected to 36 hours' strict starvation still showed an intra-gastric pH level of the order of 2 to 3, as measured with Lyphan indicator paper. Judging by the few existing investigations of the production of *pepsin and mucin* in the mouse, it would seem that these substances also are produced continuously (*10*).

In order to reduce the gastric juice production to the lowest possible resting value, the mice used in these experiments were starved for 36 hours under conditions intended to preclude coprophagia. For coprophagia is the reason given by previous workers for the presence of matter in the stomachs of rats, even several days after the withdrawal of their food (e.g. *70*), and it is most likely that this is conducive to the production of gastric juice (cf. *3*). To enclose the mice in a special "cage" during the starvation period seems an effective means of preventing coprophagia, in any case there was little or no matter in the stomachs of these mice.

However, despite the mice seeming to have a certain fasting secretion of gastric juice, it appears to be beyond doubt that the intake of food stimulates the glandular cells in the gastric mucosa to greater activity than during strict starvation (cf. *10*).

In the stimulated mice adequate preservation of the epithelial cells of the fundus glands proved even harder to attain than in the starved mice. Compared with the gastric mucosa from unstimulated animals, that from stimulated mice embedded in methacrylate after fixation at pH 8.5 exhibited pronounced ultrastructural dissimilarities. However, considering that some of the usual signs of poor preservation were often present, primarily in the zymogen cells but also, though to a lesser extent, in the other cells, it is hard to estimate the significance of such dissimilarities. After fixation at pH 7.2 the preservation was still poorer. Conversely, tissues fixed at pH 8.5 and embedded in Vestopal only rarely displayed changes suggestive of poor preservation. Hence it is highly probable that ultrastructural differences between corresponding epithelial cells in Vestopal embedded fundus glands fixed at pH 8.5

from starved and stimulated mice are primary and reflect differences in the functional state of the fundus glands.

The greater difficulty of achieving adequate preservation of the gastric mucosa from stimulated mice can be explained by the aforementioned hypotheses regarding proteolytic changes (see p. 87). The release of pepsin and hydrochloric acid is higher in stimulated animals, and the risks for a postmortal, proteolytic action on the cells of the fundus glands should therefore be greater than in starved animals.

In previous ultrastructural investigations on animals whose gastric juice production had been stimulated, signs of poor preservation apparently have been ignored to a large extent. Thus, it has been suggested that the large cytoplasmic projections from the apical cell surface (*99*), and the occurrence of areas of reduced density and the partial absence of inner membranes in some mitochondria (*186*, *187*) are primary modifications in the ultrastructural appearance of the parietal cells accompanying enhanced hydrochloric acid production. More likely, however, such changes are secondary, resulting from postmortal proteolytic processes.

## DISCUSSION OF RESULTS

### *Zymogen cells*

The outstanding ultrastructural difference between zymogen cells from starved and refed mice is observed in the Golgi apparatus. Its extent is thus distinctly greater in animals given food than in fasted ones. A great number of zymogen granules of varying size and density are always encountered in the neighborhood of the enlarged Golgi structures. As emphasized above (see p. 102), however, zymogen granules may at times be impossible to distinguish from Golgi vacuoles or large Golgi vesicles. Shibasaki (*191*) reported comparable changes in the zymogen cells from rats after refeeding.

Similar observations regarding the topographical relations between the secretory granules and the Golgi apparatus in a number of other cell types have formed the basis for several hypotheses with regard to the part played by the Golgi apparatus in the production of secretory granules. According to a hypothesis that has increasingly gained adherents in recent years, the function of the Golgi apparatus is to store and concentrate substances produced elsewhere in the cells (e.g. *36*, *61*, *63*, *159*, *197*). It has also been suggested that the Golgi apparatus may be involved in the actual synthesis of secretory products (e.g. *159*, *194*). The present author's observations in the fundus-gland cells from fasted and refed mice contribute no new arguments to this debate. On the other hand, the increase of the extent of the Golgi apparatus is very well compatible with an augmented production of secretory granules.

The diameter of the zymogen granules is smaller in the refed mice than in the fasted ones. This is probably due to a higher proportion of newly created zymogen granules. Contrary to earlier electron microscopic findings in the rat (*108*, *191*), no definite reduction in the number of zymogen granules could be observed in the present study. However, such a reduction would probably have occurred if the postprandial period had been longer (cf. p. 93).

The varying, but for the most part increased, postprandial width of the cisternae bounded by the α-cytomembranes gives rise to a considerable expansion of the volume of these cisternae. The zymogen cells of the fundus glands exhibited no parallel to the intra-cisternal granules observed in the similarly dilated cisternae of the acinar pancreatic cells in refed guinea pigs (*154*) and ethionine-treated rats (*51*). Just as has been suggested in the case of the refed guinea pigs (*157*, *158*), however, the increased volume of the intracisternal cytoplasm probably is intimately associated with the accelerated protein production of these cells. In this connection it is interesting to note that the less dense layer of the nuclear membrane is dilated in a way similar to that of the cisternae in the cytoplasm. This similarity in the patterns of reaction would seem to bear out the view that the nuclear membrane is homologous to the α-cytomembranes (cf. p. 86).

*Parietal cells*

After 30 minutes' refeeding the most prominent ultrastructural change in the parietal cells is the increased number of microvilli in the intracellular canaliculi as well as on the apical cell surface. They have a more irregular shape than in the starved animals and a more varying diameter.

The greater abundance of microvilli is apparently parallelled by some increase in the calibre of the intracellular canaliculi in the parietal cells.

These postprandial ultrastructural changes in the parietal cells—which on the whole agree with those reported by other workers (*187*, *191*)—greatly increase the area of cell surface in functional contact with the glandular lumen. Since this change is morphologically dominant it is presumably associated with the secretory function of the parietal cells.

In most parietal cells the number of vacuoles is reduced after refeeding. However, in some cells their number apparently remains unchanged. This variation in the number of vacuoles, as well as the discrepancies between earlier observations on this point, could be due to different degrees of functional activity of the examined cells. It is thus possible that some of the cells which have been studied have reacted less markedly than others to the applied stimuli. A similar explanation has also been proposed by Sedar *et al.* (*187*).

Several authors have postulated that the vacuoles serve as receptacles for the secre-

tory products of the parietal cells (*108*, *109*, *187*, *191*). The hydrochloric acid would be released by the vacuoles establishing communications with the glandular lumen or with the lumen of the intracellular canaliculi. However, using electron microscopic techniques, this theory cannot yet be tested.

No significant alteration of the number of cytoplasmic particles could be discovered in the present investigation. Nor has the writer been able to verify the statement that the mitochondrial diameter increases and the distance between the inner mitochondrial membranes grows larger in the parietal cells after stimulation (*186*, *187*). Increases in the size of vacuole-containing bodies which have been described by Hally (*75*) were observed only in sections from poorly preserved tissues.

Accordingly the parietal cells exhibit relatively minor ultrastructural changes after refeeding compared with protein-producing cells, such as zymogen cells and the acinar pancreatic cells.

The absence of major ultrastructural changes in the parietal cells after stimulation agrees with earlier observations in cells of other types presumably involved in the metabolism of the organism of water and electrolytes. Thus, despite pronounced diurnal variations in the volume of urine, most likely due to differences in tubular reabsorption, Rhodin (*168*) discerned no ultrastructural differences between the cells of the renal proximal tubules at maximal and at minimal urinary output. Similarly, when Buck *et al.* (*19*) studied the ependymal cells of the chorioid plexus they found no consistent change from normal after administration of various substances known to enhance the production of cerebrospinal fluid.

*Mucoid cells*

After refeeding the mucoid cells of the fundus glands merely exhibit minor, inconsistent ultrastructural changes (cf. *191*). Since the mucoid cells have a polymorphic appearance even in the fasting state, any ultrastructural deviations must be highly conspicuous in order to be definitely distinguished from the non-stimulated state.

However, the absence of marked ultrastructural effects of stimulation in the mucoid cells seems well compatible with their function. For, analogously with what has been shown regarding the mucoid cells of the fundus gland in other species (*3*), the amount of mucin elaborated by these cells in the mouse would seem to be only slightly affected by the type of stimulation implicit in refeeding (*10*). The alterations in cellular metabolism induced by refeeding are apparently much less pronounced for mucoid cells than for, say, the zymogen cells.

*Argyrophil cells*

The absence of postprandial ultrastructural changes in the argyrophil cells indicates that these cells lack any immediate digestive function and is compatible with their assumed endocrine function.

## SUMMARY

The ultrastructure of the fundus glands of the gastric mucosa has been studied in the mouse. Part One deals with the appearance of these glands in mice after 36 hours' fasting and Part Two with the corresponding features in mice after a 30-minute feeding period following such fasting. In Part One the morphological observations were based on a total of 25 mice, in Part Two on eight mice.

In view of the susceptibility of the gastric mucosa to develop post-mortal proteolytic changes, various procedural modifications were made in the hope of reducing the extent of such changes, mainly variations in the pH and buffering capacity of the fixation fluid and the use of two different embedding media. Certain commonly occurring structures were selected for statistical analysis of the differences that might exist between the dimensions of the respective structures after different preservation methods. The level of significance was set at 5%. The same type of analysis was also performed when comparing these structures in fasted and in refed mice. The results of the measurements are compiled in Tables I and II (pp. 114–117).


## FIXATION FLUIDS

The tissue was fixed in an ice-cold blood-isotonic 1 % osmium tetroxide solution veronal acetate buffered to either pH 7.2 or pH 8.5. In the latter case the buffering capacity towards acidity was appreciably higher (Text-fig. 2).

Among the various cells of the fundus glands, the electron microscopic appearance of the zymogen cells and of the parietal cells was studied after fixation at pH 7.2 as well as after fixation at pH 8.5. Since the mucoid cells and argyrophil cells as a rule became well preserved only after fixation at pH 8.5, the observations on the ultrastructure of these cells were confined to specimens treated in this manner.

The outstanding ultrastructural difference between methacrylate embedded fundus glands fixed at pH 7.2 and those fixed at pH 8.5 was the appreciably higher incidence of well-preserved epithelial cells in the latter case, the difference probably being due mainly to more effective inhibition of the autolytic action of pepsin at the higher pH level. After fixation at pH 8.5, the cytoplasmic ground substance was more dense and the zymogen granules often less dense than after fixation at pH 7.2. Otherwise these two groups exhibited no striking ultrastructural differences.

## EMBEDDING MEDIA

The specimens were embedded either in a methacrylate mixture or in a polyester–Vestopal W. Most of the specimens embedded in Vestopal had been fixed at pH 8.5.

Compared with methacrylate, Vestopal exhibits the higher electron optical density. The three-layered structure of most membranes appears much more distinct than in methacrylate embedded tissue. Moreover, when Vestopal is used, a variety of cellular organelles are of significantly greater size than when methacrylate has been employed. Fundamentally, however, corresponding cells embedded in these two media are ultrastructurally similar. In Vestopal embedded sections fixed at pH 8.5, the fundus-gland cells from fasted mice very rarely exhibited indications of being poorly preserved. Moreover, this preservation technique was the only one yielding satisfactory results with tissue from refed mice.

## PART I. FASTED MICE

The following account is based on observations of methacrylate embedded sections fixed at pH 7.2 so far as zymogen cells and parietal cells are concerned, and at pH 8.5 with respect to mucoid cells and argyrophil cells.

*Zymogen cells* (Figs. 2–12)

The fundus glands are built up like tubules. In the basal part of these tubules zymogen cells constitute the main component of the glandular walls. The apical surface of the zymogen cell is provided with a small number of microvilli with an average diameter of about 780 Å. The other cell surfaces are fairly even, apart from minor *interdigitations* with adjacent epithelial cells along the lateral surfaces. Both *terminal bars* and *desmosomes* are present on the lateral cell surfaces.

*Zymogen granules* with an average diameter of approximately 0.70 $\mu$ are encountered in the supranuclear and paranuclear portions of the cytoplasm. Their content is either dense and homogeneous in appearance or moderately dense and floccular. In the latter case, the distinct surface membrane of the zymogen granules is incomplete.

Scattered throughout the cytoplasm, the few *mitochondria* are often elongated and have a minor axis approximately 0.29 $\mu$ long. They contain transversely disposed inner membranes and occasional opaque particles measuring some 290 Å across.

An extensive *Golgi apparatus* is encountered in the supranuclear or paranuclear cytoplasm. It comprises a number of membranes, comparatively large vacuoles and a few vesicles. Small zymogen granules are observed in its neighborhood.

*α-Cytomembranes* disposed in pairs constitute a dominating feature in the infranuclear portion of the cytoplasm, where they are arranged in long strands, roughly parallel to the cell surface. These formations are less abundant and occur only as short strands elsewhere in the cytoplasm. A large number of maximally dense cytoplasmic *particles* with a diameter of some 130 Å are observed on and between the α-cytomembrane pairs.

Furthermore, the cytoplasm of these cells contains occasional *vacuole-containing bodies*, *dense microbodies* and *lipid droplets*.

The *nucleus* of the zymogen cell is surrounded by a three-layered membrane which at intervals exhibits discontinuities.

*Parietal cells* (Figs. 13–22)

Parietal cells are encountered along the entire length of the fundus glands, though only sporadically in the upper third of the glands. The cytoplasm is pervaded by tortuous *intracellular canaliculi* which open at the apical surface. From both the apical cell surface and the walls of the intracellular canaliculi protrude an abundance of *microvilli* with an average diameter of some 730 Å. On the lateral cell surfaces one encounters *terminal bars* and occasional *interdigitations* with adjacent epithelial cells. The basal surface is provided with a number of shallow concavities.

Smooth-surfaced *vacuoles* with a mean diameter of about 0.14 μ occur abundantly throughout the cytoplasm of the parietal cell, except in the most basal portions where they are rarer. There, as well as marginally along the lateral cell surfaces, *mitochondria* occur in large numbers. Having a minor axis averaging about 0.56 μ in length, they contain an abundance of parallelly disposed inner membranes and a few, opaque particles with a diameter of approximately 360 Å.

The *Golgi apparatus* is comparatively small in parietal cells. *α-Cytomembranes* and maximally dense cytoplasmic *particles* occur only sparsely. Among other cytoplasmic organelles, mention may be made of *vacuole-containing bodies*, *dense microbodies* and *lipid droplets*.

The *nucleus* has a three-layered surface membrane with only few discontinuities.

*Mucoid cells* (Figs. 1, 13, 19, 23–34)

Mucoid cells are found in the upper part of the fundus glands. Indeed these parts of the glandular tubule are occasionally composed solely of such cells. Their apical surface is provided with a small number of short, stubby projections. On the usually markedly undulated lateral surfaces one encounters both *terminal bars* and *desmosomes*. The lateral surfaces of the mucoid cells occasionally border against dilated intercellular spaces, here called *lacunae*, which sometimes contain very altered cellular

organelles. Mucoid cells abutting on such lacunae exhibit indications ascribed to impaired vitality.

Apically in the mucoid cells one finds a zone of varying width containing closely packed *secretory granules*. These have an average diameter of about 0.26 $\mu$ and exhibit a varying density.

Many of the *mitochondria* of the mucoid cells contain one or more round granules. The mitochondria moreover contain a few transversely disposed inner membranes and occasional opaque particles. The diameters of the mitochondria, the mitochondrial granules, and the mitochondrial particles average about 0.16 $\mu$, 0.11 $\mu$, and 340 Å respectively.

The *Golgi apparatus* consists of membranes, flat vacuoles, and a comparatively large number of vesicles. In its neighborhood one often encounters small secretory granules.

Whereas *α-cytomembranes* occur rather sparsely in the cytoplasm, the number of maximally dense cytoplasmic *particles* is great. In mucoid cells, too, a small number of *vacuole-containing bodies*, *dense microbodies*, and *lipid droplets* have been encountered. In addition *fibrillar elements* are not seldom encountered, sometimes apparently arising from the lateral cell surfaces.

The appearance of the *nucleus* is largely the same as in the zymogen cells.

*Argyrophil cells* (Figs. 35–38)

The fundus glands of the mouse have a small number of argyrophil cells. These cells have comparatively regular surfaces and do not communicate with the glandular lumen.

Scattered throughout the cytoplasm of these cells one finds numerous *secretory granules* with a diameter averaging about 0.18 $\mu$. A secretory granule comprises an annular core of high density surrounded by a narrow zone of low density which, in turn, is encircled by a surface membrane.

The *mitochondria* of the argyrophil cells are often elongated and have a minor axis averaging about 0.14 $\mu$. Mitochondrial inner membranes are sparse.

The *Golgi apparatus* is comparatively small. In its vicinity there are secretory granules which are smaller than those elsewhere in the cytoplasm and have a wider annular zone than these.

Furthermore, one observes in the cytoplasm one or more so-called *pigment bodies*. These formations usually range from 0.5 to 1.5 $\mu$ in diameter, contain a substance of high density, and are surrounded by a varying number of membrane pairs.

A small number of *α-cytomembranes*, maximally dense cytoplasmic *particles*, and irregularly shaped *vacuoles* are encountered in the argyrophil cells also.

The three-layered surface membrane of the *nucleus* exhibits a small number of discontinuities.

## PART II. REFED MICE

In this part of the investigation, the ultrastructure of fundus glands from refed mice which had been fixed at pH 8.5 and embedded in Vestopal was compared with that of similarly treated tissue from fasted mice. The following dissimilarities were noted.

*Zymogen cells* (Figs. 39–42)

The Golgi apparatus becomes markedly more extensive. Its component vacuoles are dilated and its vesicles more abundant. On average the diameter of the zymogen granules is reduced by approximately 30 per cent. The regular arrangement of the α-cytomembranes in the basal portion of the zymogen cells is lost, and the interspace between the individual membranes of a pair varies greatly. Similarly, the distance between the dense layers of the nuclear membrane varies.

*Parietal cells* (Figs. 43, 44)

In the parietal cells the microvilli on the apical cell surface and within the intracellular canaliculi become more abundant and, concomitantly, their shape grows more irregular. The incidence of cytoplasmic vacuoles declines in the majority of cells.

*Mucoid cells and argyrophil cells*

No definite postprandial changes can be observed in the mucoid cells and the argyrophil cells.

## TABLE I

MEAN DIMENSION AND RANGE OF SOME ULTRASTRUCTURAL FORMATIONS IN THE EPITHELIAL CELLS OF THE FUNDUS GLANDS OF FASTED MICE

| Formation | Mean | Range | No. of measurements | No. of cells | No. of mice |
|---|---|---|---|---|---|
| I. ZYMOGEN CELL | | | | | |
| a. *Fixed at pH 7.2. Embedded in methacrylate* | | | | | |
| Apical three-layered plasma membrane, thickness | 77 Å | 46–115 Å | 60 | 12 | 5 |
| Distance between centers of dense layers in above | 54 Å | 37– 80 Å | 50 | 9 | 4 |
| Thickness of lateral plasma membrane + width of lateral intercellular space | 131 Å | 91–174 Å | 45 | 10 | 3 |
| Lateral intercellular space, width | 77 Å | 58–105 Å | 45 | 10 | 3 |
| Microvillus, diameter | 776 Å | See Table II | | | |
| Dense zymogen granule, diameter | 0.68 μ | | | | |
| Less dense zymogen granule, diameter | 0.73 μ | | | | |
| Mitochondrial minor diameter | 0.29 μ | | | | |
| Mitochondrial three-layered outer membrane, thickness | 122 Å | 89–174 Å | 52 | 8 | 4 |
| Distance between centers of dense layers in above | 87 Å | 57–114 Å | 42 | 8 | 4 |
| Mitochondrial three-layered inner membrane, thickness | 126 Å | 80–180 Å | 34 | 10 | 5 |
| Distance between centers of dense layers in above | 96 Å | 67–126 Å | 34 | 10 | 5 |
| Mitochondrial particle, diameter | 292 Å | 184–387 Å | 46 | 10 | 3 |
| Golgi membrane pair, total thickness | 158 Å | 128–199 Å | 39 | 8 | 4 |
| Distance between centers of membranes in above | 112 Å | 75–148 Å | 42 | 7 | 4 |
| Cytoplasmic particle, diameter | 128 Å | 91–211 Å | 48 | 7 | 4 |
| Vacuole-containing body, diameter | 0.24 μ | 0.15–0.41 μ | 17 | 10 | 4 |
| Vacuole in above, diameter | 384 Å | 252–497 Å | 16 | 7 | 3 |
| Nuclear particle, diameter | 132 Å | 80–186 Å | 61 | 10 | 7 |
| b. *Fixed at pH 8.5. Embedded in methacrylate* | | | | | |
| Microvillus, diameter | 833 Å | See Table II | | | |
| Zymogen granule, diameter | 0.65 μ | | | | |
| Mitochondrial minor axis | 0.30 μ | | | | |
| c. *Fixed at pH 8.5. Embedded in Vestopal* | | | | | |
| Microvillus, diameter | 920 Å | See Table II | | | |
| Zymogen granule, diameter | 1.04 μ | | | | |
| Mitochondrial minor axis | 0.37 μ | | | | |
| II. PARIETAL CELL | | | | | |
| a. *Fixed at pH 7.2. Embedded in methacrylate* | | | | | |
| Apical and canalicular three-layered plasma membrane, thickness | 72 Å | 48–93 Å | 30 | 11 | 5 |
| Distance between centers of dense layers in above | 53 Å | 42–68 Å | 31 | 11 | 4 |
| Thickness of lateral plasma membrane + width of lateral intercellular space | 135 Å | 96–166 Å | 35 | 12 | 5 |
| Lateral intercellular space, width | 79 Å | 58–118 Å | 35 | 8 | 5 |
| Microvillus, diameter | 727 Å | See Table II | | | |
| Vacuole, diameter | 0.14 μ | | | | |

Table I (continued)

| Formation | Mean | Range | No. of measurements | No. of cells | No. of mice |
|---|---|---|---|---|---|
| Surface membrane of vacuole, thickness | 66 Å | 48–96 Å | 35 | 10 | 4 |
| Mitochondrial minor axis | 0.56 μ | See Table II | | | |
| Mitochondrial three-layered outer membrane, thickness | 102 Å | 69–136 Å | 38 | 13 | 4 |
| Distance between centers of dense layers in above | 77 Å | 52–109 Å | 38 | 13 | 4 |
| Mitochondrial three-layered inner membrane, thickness | 132 Å | 97–193 Å | 53 | 8 | 4 |
| Distance between centers of dense layers in above | 102 Å | 78–145 Å | 53 | 8 | 4 |
| Mitochondrial particle, diameter | 356 Å | 214–496 Å | 60 | 10 | 5 |
| Cytoplasmic particle, diameter | 124 Å | 79–183 Å | 60 | 6 | 4 |
| Vacuole-containing body, diameter | 0.38 μ | 0.23–0.85 μ | 40 | 11 | 6 |
| Vacuole in above, diameter | 420 Å | 295–656 Å | 32 | 11 | 4 |
| Nuclear particle, diameter | 127 Å | 80–168 Å | 45 | 11 | 7 |
| b. *Fixed at pH 8.5. Embedded in methacrylate* | | | | | |
| Microvillus, diameter | 806 Å | See Table II | | | |
| Vacuole, diameter | 0.14 μ | | | | |
| Mitochondrial minor axis | 0.47 μ | | | | |
| c. *Fixed at pH 8.5. Embedded in Vestopal* | | | | | |
| Apical and canalicular three-layered plasma membrane, thickness | 83 Å | 65–105 Å | 25 | 6 | 5 |
| Distance between centers of dense layers in above | 64 Å | 49–72 Å | 25 | 6 | 5 |
| Microvillus, diameter | 959 Å | See Table II | | | |
| Vacuole, diameter | 0.13 μ | | | | |
| Vacuolar three-layered surface membrane, thickness | 74 Å | 57–98 Å | 33 | 5 | 4 |
| Distance between centers of dense layers in above | 54 Å | 41–78 Å | 33 | 5 | 4 |
| Mitochondrial minor axis | 0.54 μ | See Table II | | | |
| III. Mucoid cell | | | | | |
| a. *Fixed at pH 8.5. Embedded in methacrylate* | | | | | |
| Apical plasma membrane, thickness | 74 Å | 53–94 Å | 30 | 8 | 5 |
| Thickness of lateral plasma membrane + width of lateral intercellular space | 138 Å | 107–201 Å | 40 | 9 | 6 |
| Lateral intercellular space, width | 84 Å | 59–143 Å | 40 | 13 | 6 |
| Secretory granule, diameter | 0.33 μ | See Table II | | | |
| Mitochondrial minor axis | 0.16 μ | | | | |
| Mitochondrial three-layered outher membrane, thickness | 105 Å | 84–130 Å | 32 | 10 | 4 |
| Distance between centers of dense layers in above | 74 Å | 60–101 Å | 32 | 10 | 4 |
| Mitochondrial three-layered inner membrane, thickness | 129 Å | 96–161 Å | 36 | 11 | 4 |
| Distance between centers of dense layers in above | 99 Å | 72–132 Å | 36 | 11 | 4 |
| Mitochondrial granule, diameter | 0.11 μ | 0.06–0.19 μ | 39 | 13 | 5 |
| Mitochondrial particle, diameter | 344 Å | 115–516 Å | 40 | 13 | 7 |
| Cytoplasmic particle, diameter | 137 Å | 96–173 Å | 84 | 9 | 4 |
| Nuclear particle, diameter | 142 Å | 88–193 Å | 45 | 10 | 5 |
| b. *Fixed at pH 8.5. Embedded in Vestopal* | | | | | |
| Secretory granule, diameter | 0.27 μ | See Table II | | | |
| Mitochondrial minor axis | 0.21 μ | | | | |

Table I (continued)

| Formation | Mean | Range | No. of measurements | No. of cells | No. of mice |
|---|---|---|---|---|---|
| IV. ARGYROPHIL CELL | | | | | |
| a. *Fixed at pH 8.5. Embedded in methacrylate* | | | | | |
| Thickness of lateral plasma membrane + width of lateral intercellular space | 154 Å | 102–187 Å | 27 | 2 | 2 |
| Lateral intercellular space, width | 94 Å | 71–155 Å | 22 | 2 | 2 |
| Secretory granule, diameter | 0.18 μ | 0.09–0.27 μ | 55 | 2 | 2 |
| Thickness of surface membrane + width of annular zone of secretory granule | 116 Å | 77–157 Å | 44 | 1 | 1 |
| Annular zone of secretory granule, width | 80 Å | 48–128 Å | 44 | 1 | 1 |
| Mitochondrial minor axis | 0.14 μ | 0.09–0.20 μ | 82 | 2 | 2 |
| Mitochondrial three-layered outer membrane, thickness | 114 Å | 91–143 Å | 40 | 1 | 1 |
| Distance between centers of dense layers in above | 85 Å | 61–113 Å | 40 | 1 | 1 |
| Mitochondrial three-layered inner membrane, thickness | 148 Å | 101–214 Å | 25 | 1 | 1 |
| Distance between centers of dense layers in above | 108 Å | 81–178 Å | 25 | 1 | 1 |
| b. *Fixed at pH 8.5. Embedded in Vestopal* | | | | | |
| Secretory granule, diameter | 0.18 μ | 0.09–0.27 μ | 55 | 4 | 4 |
| Mitochondrial minor axis, thickness | 0.14 μ | 0.08–0.22 μ | 30 | 4 | 4 |

TABLE II

MEAN DIMENSION AND STANDARD DEVIATIONS OF SELECTED ULTRASTRUCTURAL FORMATIONS IN THE EPITHELIAL CELLS OF THE FUNDUS GLANDS OF FASTED AND REFED MICE.[a]

| Formation | Mean | S.D. within cells | S.D. between cells | S.D. between mice | No. of cells | No. of measurements |
|---|---|---|---|---|---|---|
| I. ZYMOGEN CELL. FASTED MICE | | | | | | |
| (*a*) *Fixed at pH 7.2. Embedded in methacrylate* | | | | | | |
| Microvillus, diameter | 776 Å | 87 | 218 | 324 | 12 | 50 |
| Dense zymogen granule, diameter | 0.68 $\mu$ | 0.146 | 0.126 | 0.589 | 11 | 52 |
| Less dense zymogen granule, diameter | 0.73 $\mu$ | 0.186 | 0.262 | 0.109 | 12 | 60 |
| Mitochondrial minor axis | 0.29 $\mu$ | 0.059 | 0.104 | 0.122 | 12 | 47 |
| (*b*) *Fixed at pH 8.5. Embedded in methacrylate* | | | | | | |
| Microvillus, diameter | 833 Å | 108 | 265 | 502 | 11 | 49 |
| Zymogen granule, diameter | 0.65 $\mu$ | 0.153 | 0.144 | 0.509 | 12 | 59 |
| Mitochondrial minor axis | 0.30 $\mu$ | 0.057 | 0.050 | 0.235 | 12 | 41 |
| (*c*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Microvillus, diameter | 920 Å | 135 | 361 | 155 | 12 | 52 |
| Zymogen granule, diameter | 1.04 $\mu$ | 0.219 | 0.472 | 0.443 | 12 | 59 |
| Mitochondrial minor axis | 0.37 $\mu$ | 0.065 | 0.079 | 0.070 | 12 | 45 |
| II. ZYMOGEN CELL. REFED MICE | | | | | | |
| (*a*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Microvillus, diameter | 973 Å | 104 | 213 | 531 | 12 | 56 |
| Zymogen granule, diameter | 0.75 $\mu$ | 0.231 | 0.308 | 0.354 | 12 | 58 |
| Mitochondrial minor axis | 0.37 $\mu$ | 0.057 | 0.109 | 0.059 | 12 | 59 |
| III. PARIETAL CELL. FASTED MICE | | | | | | |
| (*a*) *Fixed at pH 7.2. Embedded in methacrylate* | | | | | | |
| Microvillus, diameter | 727 Å | 99 | 199 | 72 | 12 | 60 |
| Vacuole, diameter | 0.14 $\mu$ | 0.023 | 0.045 | 0.073 | 12 | 60 |
| Mitochondrial minor axis | 0.56 $\mu$ | 0.080 | 0.090 | 0.399 | 12 | 59 |
| (*b*) *Fixed at pH 8.5. Embedded in methacrylate* | | | | | | |
| Microvillus, diameter | 806 Å | 135 | 278 | 189 | 12 | 58 |
| Vacuole, diameter | 0.14 $\mu$ | 0.019 | 0.063 | 0.054 | 12 | 60 |
| Mitochondrial minor axis | 0.47 $\mu$ | 0.100 | 0.186 | 0.262 | 12 | 58 |
| (*c*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Microvillus, diameter | 959 Å | 156 | 159 | 209 | 12 | 57 |
| Vacuole, diameter | 0.13 $\mu$ | 0.021 | 0.042 | 0.025 | 12 | 60 |
| Mitochondrial minor axis | 0.54 $\mu$ | 0.111 | 0.159 | 0.309 | 12 | 57 |
| IV. PARIETAL CELL. REFED MICE | | | | | | |
| (*a*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Microvillus, diameter | 969 Å | 170 | 254 | 519 | 12 | 59 |
| Vacuole, diameter | 0.14 $\mu$ | 0.021 | 0.034 | 0.045 | 12 | 58 |
| Mitochondrial minor axis | 0.52 $\mu$ | 0.099 | 0.185 | 0.195 | 12 | 60 |
| V. MUCOID CELL. FASTED MICE | | | | | | |
| (*a*) *Fixed at pH 8.5. Embedded in methacrylate* | | | | | | |
| Secretory granule, diameter | 0.33 $\mu$ | 0.056 | 0.120 | 0.236 | 12 | 57 |
| Mitochondrial minor axis | 0.16 $\mu$ | 0.031 | 0.048 | 0.180 | 11 | 51 |
| (*b*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Secretory granule, diameter | 0.27 $\mu$ | 0.051 | 0.088 | 0.107 | 11 | 50 |
| Mitochondrial minor axis | 0.21 $\mu$ | 0.045 | 0.050 | 0.171 | 12 | 57 |
| VI. MUCOID CELL. REFED MICE | | | | | | |
| (*a*) *Fixed at pH 8.5. Embedded in Vestopal* | | | | | | |
| Secretory granule, diameter | 0.27 $\mu$ | 0.037 | 0.076 | 0.315 | 11 | 50 |
| Mitochondrial minor axis | 0.21 $\mu$ | 0.039 | 0.063 | 0.188 | 12 | 59 |

[a] Each formation was measured in three animals. Within each animal the measurements were as a rule performed on each of 5 corresponding organelles in each of 4 cells.

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